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Office of Pollution Prevention and Toxics
Room G-099
Environmental Protection Agency
401 M Street, S.W.
Washington, D.C. 20460

Re: OPPTS-42187A; FRL-4869-1

Dear Sirs:

Enclosed, as requested, are three copies of the comments of the HAP Task Force on the proposed HAP test rule (61 Fed. Reg. 33178, as amended at 62 Fed. Reg. 67466). Three copies of the Appendix to the comments are also included.

The HAP Task Force appreciates the opportunity to comment on the proposed rule.

Sincerely,

W. Caffey Norman, III

Enclosures

CONTAINS NO CBI

BEFORE THE ENVIRONMENTAL PROTECTION AGENCY

COMMENTS ON PROPOSED TEST RULE FOR HAZARDOUS AIR POLLUTANTS

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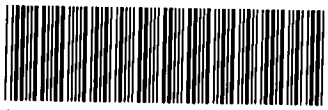

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June 22, 1998

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COMMENTS ON PROPOSED TEST RULE FOR HAZARDOUS AIR POLLUTANTS

I. INTRODUCTION

The HAP Task Force appreciates this opportunity to provide comments in response to the proposal by the Environmental Protection Agency (EPA) to require inhalation testing for 21 (now 20) hazardous air pollutants (HAPs) under Section 4 of the Toxic Substances Control Act (TSCA).[1] The proposed test rule would require testing for a number of endpoints, including acute toxicity, immunotoxicity, neurotoxicity, carcinogenicity, and developmental and reproductive toxicity. The HAP Task Force is a consortium of manufacturers of ethylene dichloride (EDC), vinylidene chloride (VDC), and 1,1,2-trichloroethane, three of the HAPs that are the subject of the proposed test rule.

The HAP Task Force appreciates the efforts that EPA has made to provide for alternative testing proposals focusing on use of existing oral data and pharmacokinetic (PK) modeling in lieu of conducting new studies by inhalation. The HAP Task Force submitted such proposals for alternative testing for EDC and 1,1,2-trichloroethane, and has replied separately to EPA's responses to these proposals. The Task Force has made clear that those submissions and its ongoing discussions with EPA staff concerning possible enforceable consent agreements (ECAs) are without prejudice to its position, elaborated in these comments, that the proposed test requirements for EDC, VDC, and 1,1,2-trichloroethane are unauthorized by statute and unwise as a matter of policy. They would impose a significant expense on the producers of these chemicals while yielding negligible public health benefits. Members of the HAP Task Force also belong to the Chemical Manufacturers Association (CMA), and the HAP Task Force supports and incorporates by reference the generic comments submitted by CMA on the proposed test rule.

[1] 61 Fed. Reg. 33178 (June 26, 1996), subsequently extended at 61 Fed. Reg. 67516, 62 Fed. Reg. 9142, 62 Fed. Reg. 14850, 62 Fed. Reg. 29318, 62 Fed. Reg. 37833, 62 Fed. Reg. 50546, 62 Fed. Reg. 63299, amended and extended at 62 Fed. Reg. 67467, and further extended at 63 Fed. Reg. 5915 and 63 Fed. Reg. 19694.

II. THE BASIS FOR THE TEST RULE IS SUSPECT

A. The Statutory Findings for EDC, VDC, and 1,1,2-Trichloroethane Cannot Be Supported

Section 4 of TSCA authorizes EPA to require testing for HAPs if it finds that the following conditions are met: (A) that the manufacture, distribution in commerce, processing, use, or disposal of a chemical substance or mixture, or any combination of such activities, may present an unreasonable risk of injury to health or the environment; or (B) that a chemical substance or mixture is or will be produced in substantial quantities and (i) it enters or may reasonably be expected to enter the environment in substantial quantities, or (ii) there is or may be significant or substantial human exposure to such substance or mixture.[2] To support either finding, EPA must show that there are insufficient data and experience upon which the effects of the manufacture, distribution in commerce, processing, use, or disposal of such substance or mixture or of any combination of such activities on health or the environment can reasonably be predicted.[3] EPA purports to justify the proposed test rule for EDC, VDC, and 1,1,2-trichloroethane under both Section 4(a)(1)(A) (the "A" finding) and Section 4(a)(1)(B) (the "B" finding). As discussed below, the "A" findings are inadequate for EDC, VDC, and 1,1,2-trichloroethane. As to the "B" findings, there are serious questions as to EPA's use of strictly numerical criteria. Even assuming that these criteria may be used, however, it is apparent that exposure to 1,1,2-trichloroethane and VDC do not meet the standard for requiring testing under Section 4(a)(1)(B).

1. The "A" Findings

Section 4(a)(1)(A) requires EPA to determine that manufacture, distribution, processing, use, or disposal of the substance may present an unreasonable risk of injury to health. This determination cannot be made in the abstract; rather, it requires an analysis of human exposure to the substance and the potential for injury at such exposure levels. The D.C. Circuit has held that EPA must "correlate the suspected toxicity of a substance with the

[2] 15 U.S.C. § 2603(a).

[3] *Id.*

suspected levels of exposure,"[4] and that a test rule is warranted only "when there is a more-than-theoretical basis for suspecting that some amount of exposure occurs and that the substance is sufficiently toxic *at that exposure level* to present an 'unreasonable risk of injury to health.'"

For EDC, VDC, and 1,1,2-trichloroethane, EPA has completely failed to show a relationship between expected exposure scenarios and the health effects endpoints for which it would require testing. In the case of 1,1,2-trichloroethane, for example, EPA must show not that the substance may pose a risk of reproductive, developmental, or other effects for which it proposes testing at some imagined human exposure, but at the levels approaching the limits of detection at which actual human exposure occurs.

Another flaw in EPA's approach is its failure adequately to identify why it thinks these HAPs may pose the risks identified.[5] By far the most significant defect in the "A" findings, however, is EPA's effort to transform a single finding of an "unreasonable risk of injury to health" (*i.e.*, carcinogenicity) into *carte blanche* authority to require *any* testing necessary to address unanswered questions about the effects of the chemical substance. The Task Force submits that Congress did not intend for Section 4 of TSCA to authorize EPA to impose regulatory requirements costing vast amounts of money to investigate unrelated health effects of a substance just because it may pose a carcinogenic risk. Again using the example of 1,1,2-trichloroethane, it is inexplicable that EPA would propose to require developmental and reproductive toxicity testing on the basis of an "A" finding based on oncogenicity, and liver, kidney, and neurotoxicity.

2. The "B" Findings

For all 20 HAPs, the proposed test rule is also based on Section 4(a)(1)(B) -- production in substantial quantities and substantial human exposure.[6] It is clear, however, that human exposure to 1,1,2-trichloroethane and VDC are not "substantial" for purposes of TSCA under the guidelines set by EPA itself.

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[4] *CMA v. EPA*, 859 F.2d 977, 995 (D.C. Cir. 1988) (emphasis added).

[5] *See* TSCA Section 4(a) Statutory Findings, 61 Fed. Reg. at 33190-93.

[6] 61 Fed. Reg. at 33193.

On May 14, 1993, EPA published a statement of policy, known as "the B Policy," articulating the standards and criteria for implementing its authority to require testing under the substantial production, release, and exposure provisions of TSCA.[7] The Agency set forth as general benchmarks for "substantial exposure" for purposes of the statute exposure of 100,000 members of the public, 10,000 consumers, or 1,000 workers.[8] In the case of 1,1,2-trichloroethane and VDC, these threshold figures are not met.

In the preamble to the proposed rule and its supporting documents, EPA presents no data, not even estimates, regarding exposure of the general population to 1,1,2-trichloroethane. Instead, the primary basis for finding substantial exposure to 1,1,2-trichloroethane is EPA's assertion that 1,036 workers were exposed to it. This figure is based solely on an estimate from the National Occupational Exposure Survey (NOES) conducted by the National Institute of Occupational Safety and Health (NIOSH) from 1981 to 1983.[9] This fifteen-year old database was of dubious accuracy at the time it was conducted, and certainly should not be considered a reliable indicator of current workplace exposure.

The NOES survey sample has been criticized for including only one percent of the 500,000 establishments listed in the Dun and Bradstreet market identifier file as of 1980.[10] In addition, NIOSH sampled large facilities more often than smaller ones, and the smaller ones that it did sample were generally not representative of typical facilities. The results of such an incomplete sampling are subject to question, and the value of the data compiled is limited.

Yet another flaw in the NOES survey was its failure to obtain qualitative information, such as the manner in which employees were exposed to the chemicals, the duration and concentration levels of exposure, or whether protective measures reduced exposure levels. Instead of detailing these circumstances, the NOES survey simply designates exposures as "part-time" or "full-time." One can only wonder whether EPA would be accepting of a

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[7] 58 Fed. Reg. 28736 (May 14, 1993).

[8] 58 Fed. Reg. at 28742.

[9] Environmental Protection Agency, *TSCA Section 4 Findings For 21 Hazardous Air Pollutants: A Supporting Document for Proposed Hazardous Air Pollutants Test Rule* (June 25, 1996) (*"Section 4 Findings"*), p. 75. The details of the 1981-1983 NOES survey appear in the docket in a NIOSH computer printout dated March 29, 1989.

[10] Deems A. Buell *et al.*, "An Assessment of the National Occupational Exposure Survey."

manufacturer-sponsored survey that failed to include large segments of the relevant population, and lacked qualitative data. In making a factual finding to justify testing requirements, EPA should subject itself and its data to the same scrutiny that the industry must meet.

The fifteen-year-old NOES approximation of workplace exposure, regardless of the questions as to its accuracy in 1983, does not reflect the number of workers exposed to 1,1,2-trichloroethane at this time. Due to workforce reductions in the chemical and processing industries, the number is now far lower. The HAP Task Force conducted a survey and determined that across all the firms that manufacture and process 1,1,2-trichloroethane, no more than 476 workers are even *potentially* exposed to 1,1,2-trichloroethane.[11] Of these, at least 83, while potentially exposed, are not actually exposed to 1,1,2-trichloroethane at detectable levels, and an additional 36 workers are included only because it is possible that they might be exposed to 1,1,2-trichloroethane byproduct on an irregular basis as a result of EDC production processes.

Similarly, in the case of VDC, the only numbers for exposure that EPA could produce in its "B" finding were based on the 1981-1983 NOES survey, which estimated that 2,675 workers were exposed to VDC. As noted above, the accuracy and methodology of the NOES survey was subject to significant criticism at the time it was conducted, and in any event is now fifteen years old, and does not reflect the current state of the industry. At the present time, the only U.S. manufacturers of VDC are The Dow Chemical Company and PPG Industries. These two manufacturers have done a survey of their own operations and those of their customers who use VDC, and estimate that the total number of workers potentially exposed is 710, well below the 1,000-worker threshold.[12] Even among these workers, the potential for exposure is small, because VDC is used in closed systems in the production of other products. For those specific tasks where direct VDC exposure is possible, employees utilize respirators or other breathing apparatus devices.

Aside from the fifteen-year old NOES numbers, EPA provides no other estimates of human exposure to VDC, except to note that "the general population may be exposed from

[11] Table A, attached.

[12] Table B, attached.

emissions from facilities involving the manufacturing, use, and processing of VDC."[13] The HAP exposure profile for VDC prepared in 1995 indicates, however, that its ambient air levels are extremely low.[14] A review of 325 samples in urban and suburban areas of the United States found that median, 75th percentile, and maximum concentrations of VDC were 0.0050, 0.0075, and 0.14 ppb respectively. In two rural areas, concentrations were less than 0.005 ppb and 0.065 ppb. Of approximately 300 breath samples taken in Elizabeth and Bayonne, New Jersey, only twelve percent contained quantifiable levels of VDC at 0.2-2 $\mu g/m^3$. Even in "source dominated areas," the median and maximum concentrations were 3.6 and 6.7 ppb respectively. In two cities where VDC was manufactured -- Freeport, Texas and Lake Charles, LA -- the mean concentrations were 0.13 ppb and 6.7 ppb respectively. These low levels of ambient air concentration belie EPA's bald assertion of substantial exposure among the general population.

In light of these hard data, EPA's justifications for imposing testing requirements for 1,1,2-trichloroethane and VDC do not withstand scrutiny. The HAP Task Force recognizes that EPA has discretion to find substantial exposure in situations where the quantitative numerical thresholds of the B Policy are not met, if additional factors exist. There is no indication, however, that EPA based its decision on any additional factors. The only explanation provided in the background documents for the proposed test rule is the perfunctory invocation of the 1981-1983 NOES estimates of worker exposure and the vague possibility of exposure among the general population. If these are shown to be erroneous -- as they have been by the more recent survey data submitted by the HAP Task Force -- EPA is without authority to impose the testing requirements for 1,1,2-trichloroethane and VDC.

3. Both Findings

To support either an "A" or a "B" finding, EPA must show that there are "insufficient data and experience" to determine or predict the effects of a chemical on human health and the environment. The HAP test rule is predicated on the assertion that the data to be required are

[13] *Section 4 Findings*, p. 83.

[14] Syracuse Research Corporation, Environmental Science Center, *Exposure Profiles for HAPs - Group 1*, (August 1995), p. 4.

necessary for EPA to conduct residual risk analyses. EPA has not described how it will conduct residual risk analyses, even though Section 112(f)(1) of the Clean Air Act requires it to do so by November 1996. In any event, Section 112(f)(2) of the Act requires EPA, within eight years of adopting technology-based standards, to adopt additional standards protecting public health with an ample margin of safety if those technology-based standards have not reduced excess cancer risk to the individual most exposed to known or suspected carcinogenic emissions from a source to a level of less than 1 in a million (1×10^{-6}).

Existing data for EDC, VDC, and 1,1,2-trichloroethane have been judged adequate by EPA to develop unit risk estimates of carcinogenic potency. As discussed below, it is extremely unlikely that any other health effect would cause the residual risk determined by EPA from sources of these HAPs to be higher than the estimated carcinogenic risk, and therefore result in more stringent emissions standards than those that will be based on potential carcinogenicity. A general statement by EPA as to why it considers portions of the toxicological data base for these compounds inadequate cannot justify *this* test rule unless the inadequacy will in some way be relevant to the residual risk determination.

B. EPA Has Not Realistically Assessed Emissions Patterns and Actual Risks to Human Health

In developing the proposed test rule, EPA has not factored the likelihood of human exposure into its considerations. This is a fundamental error, particularly in light of the Agency's statement that the primary purpose of the test rule is to develop data for residual risk determinations under the Clean Air Act. EPA appears to have made no attempt to determine general population exposures from industrial releases, or to gauge the impact that the ongoing implementation of maximum achievable control technology (MACT) standards under Section 112 of the Clean Air Act will have on those releases. The potential for exposure as a result of industrial releases varies greatly among the HAPs, but EPA pays it scant attention. For those HAPs produced primarily as byproducts (*i.e.*, 1,1,2-trichloroethane), used as feedstock (*i.e.*, EDC, VDC), or both, emissions will be very low even though production may be high. Low emissions mean that ambient air concentrations are low and exposure is minimal.

Because EPA fails to consider emissions patterns and exposure levels, its finding that existing data are insufficient to enable it to determine whether the HAPs present an unreasonable risk to health or the environment is flawed. The proposed test rule would require testing where it is not warranted by the exposure data, and would mandate submission of information that in all likelihood will be completely irrelevant to any residual risk determination. If the emissions patterns and exposure levels for EDC, VDC, and 1,1,2-trichloroethane are taken into account, the existing data, combined with exposure models, are clearly sufficient for EPA's purposes.

1. 1,1,2-Trichloroethane

a. The Low Exposure Profile of 1,1,2-Trichloroethane Enables EPA To Base Determinations on Existing Data

Exposure to 1,1,2-trichloroethane is very low. It is produced almost entirely as a byproduct in the production of EDC or as a captive intermediate in the production of VDC. Only one manufacturer markets 1,1,2-trichloroethane, and it sells less than 3 million pounds to fewer than 10 customers. The Agency for Toxic Substances and Disease Registry (ATSDR) examined ambient air concentrations of 1,1,2-trichloroethane and concluded that "[w]here 1,1,2-trichloroethane is found, levels appear to be about 10-50 ppt [parts per trillion]."[15] This low environmental exposure is consistent with available information on the product's use. ATSDR found that "[n]o use with significant consumer and general population exposures has been identified."[16]

The minimal exposure profile of 1,1,2-trichloroethane and the existing information as to its health effects indicate that EPA can -- instead of imposing nearly $4 million in test requirements on industry -- conduct a screening analysis and determine that the risk to health and the environment is minute based on the existing data. In fact, EPA has performed such risk assessments for 1,1,2-trichloroethane in the past. For example, the Agency's Integrated Risk Information System (IRIS) provides a unit risk estimate (or cancer potency) for

[15] Agency for Toxic Substances and Disease Registry, *Toxicological Profile for 1,1,2-Trichloroethane* (1989), p. 63 (Appendix, Tab 1).

[16] *Id.*, p. 61.

1,1,2-trichloroethane of 1.6 x 10^{-5}. In drinking water, the maximum contaminant level goal (MCLG) is 3 μg/l, which according to EPA corresponds to a theoretical cancer risk of 1 x 10^{-5}, and the maximum contaminant level (the enforceable limit) is 5 μg/l, the same as for organic contaminants which have an MCLG of zero. The data supporting these determinations, when combined with what is known about the exposure profile for 1,1,2-trichloroethane, provide sufficient information for EPA to conduct a screening analysis and make residual risk determinations. Expensive testing for other endpoints is unnecessary.

A specific illustration of this point is provided by an evaluation of air concentrations of 1,1,2-trichloroethane at the fenceline of one of the fewer than 20 U.S. facilities where EDC is produced, specifically, the Dow Chemical Company facility in Lake Jackson, Texas. Actual monitoring data immediately outside the facility showed that out of 306 samples, 97% were non-detects for 1,1,2-trichloroethane at 0.55 $\mu g/m^3$. Only three values exceeded the detection limit. Put another way, only three samples exceeded an upper bound potential cancer risk of 9 x 10^{-6} (using the EPA unit risk estimate of 1.6 x 10^{-5}). In light of the negligible risk of cancer using a nonthreshold model, it seems inconceivable that significant reproductive or developmental toxicity or other health effects would result from current or future anticipated exposure scenarios for 1,1,2-trichloroethane.

b. Sufficient Information Is Available on the Endpoints for which Testing Would Be Required

The proposed test rule would require the following tests for inhalation exposure to 1,1,2-trichloroethane: acute toxicity, subchronic toxicity, developmental toxicity, reproductive toxicity, neurotoxicity, carcinogenicity, *in vivo* cytogenicity, and immunotoxicity. The estimated cost for these tests totals approximately $3.8 million. A review of the data currently available on 1,1,2-trichloroethane reveals that much of this testing is unnecessary. Oral studies have already been performed in four of the eight areas of testing listed by EPA -- subchronic toxicity, neurotoxicity, carcinogenicity and immunotoxicity. These can serve as the basis for route-to-route extrapolation, a much more cost-effective means of determining the safety of inhalation exposure. Of the remaining four areas in which EPA has proposed testing,

only limited inhalation studies (if any) will be necessary to evaluate acute toxicity, and developmental and reproductive studies and *in vivo* cytogenicity studies should not be necessary at all. Accordingly, EPA's needs can be satisfied by the existing data, supplemented with some limited modeling as necessary.

i. Acute Toxicity

While EPA could not identify sufficient inhalation or oral studies to assess acute toxicity, little additional information is necessary due to the physical and chemical properties of 1,1,2-trichloroethane. EPA has classified 1,1,2-trichloroethane as a Category 3 inhaled chemical -- water soluble/perfusion limited. Consequently, effects on the lung (portal of entry) are unlikely. This is borne out by studies showing little or no damage to the lungs following inhalation exposure to 1,1,2-trichloroethane, and that 1,1,2-trichloroethane does not accumulate in the lungs following inhalation exposure, but rather distributes rapidly to other tissues.[17] The only justification for collecting acute toxicity data (including for the lung) for 1,1,2-trichloroethane would be to support the development and validation of PBPK models to be used for other endpoints.

ii. Subchronic Toxicity

Available oral data, summarized below and in the attached Table 1, provide adequate information for a complete assessment as to the subchronic effects of 1,1,2-trichloroethane:

- *White et al. (1985)* (Appendix, Tab 2) - Groups of 32-48 male and 32-48 female CD-1 mice were exposed to doses of 0, 4.4, 46, or 305 mg/kg-day (males), or 0, 3.9, 44, or 384 (females) 1,1,2-trichloroethane in the drinking water for 90 days. Drinking water consumption and body weight gain were reduced in concentration-dependent manner in male mice, but not in female mice. Absolute liver and kidney weights were decreased in male mice receiving 46 or 305 mg/kg-day; however, this was not observed when organ weights were expressed relative to body weight. Liver weights were significantly increased (absolutely and relatively) in female mice exposed to the highest dose. Absolute spleen and kidney weights were also elevated in this group. Hematological changes in male mice did not appear to be

[17] Bonnet, *et al., Determination of the Median Lethal Concentration of Principal Chlorinated Aliphatic Hydrocarbons in the Rat*, Arch. Mal. Prof. Med. Trav. Secur. Soc. 41:317-321 (1980) (French); Takahara, K., Experimental Studies on Toxicity of Trichloroethane: Part 1, *Organ Distribution of 1,1,1- and 1,1,2-Trichloroethanes in Exposed Mice*, Okayama Igakkai Zasshi 98:1079-1090 (1986) (Japanese).

significant. However, hemoglobin and hematocrit values were significantly decreased in female mice exposed to the highest dose. Fibrinogen was increased in all exposed females, however this was not in a dose-dependent manner. Prothrombin time was significantly decreased in a dose-dependent manner in female mice exposed to 44 or 384 mg/kg-day. In male mice, serum cholesterol and serum alkaline phosphatase activity was significantly increased at the highest dose. In female mice, serum cholesterol and SGPT activity was significantly increased at the highest dose. Liver glutathione was significantly decreased in a dose-dependent manner in males exposed to the two highest doses, but was increased in females exposed to the highest dose. The dose-response information from female mice were used to base a LOAEL and NOAEL since they present a more consistent pattern of toxicity. This study identifies a LOAEL of 384 and a NOAEL of 44 mg/kg-day for increased liver weight, serum cholesterol, and SGPT activity, and decreased (10%) hemoglobin and hematocrit in female mice.

- *NCI (1978)* (Appendix, Tab 3) - Groups of 50 male and 50 female rats (Osborne-Mendel) were exposed to each of two dose levels via corn oil gavage, 5 days/week for 78 weeks. Two control groups (vehicle and untreated) of 20 animals/sex were also included in the study. Initial doses of 0, 30, and 70 mg/kg-day were raised to 0, 50, and 100 mg/kg-day after week 8 since animals seemed to tolerate exposure. Time-weighted average doses of 0, 46, and 92 mg/kg-day were calculated by the study authors. There was no appreciable difference in mortality or body weight gain from controls. However, high mortality was noted in the vehicle control group. During the first 6 months, the incidence of clinical signs (*i.e.*, appearance and behavior changes) were comparable between exposed and control animals. However, after 6 months, exposed animals exhibited a higher frequency of hunched appearance, rough fur, urine stains, wheezing, dyspnea, and squinted eye (sometimes with reddish exudate). Histopathological examinations revealed no significant differences between exposed and control animals for noncancer effects in any tissue site (including the respiratory tract). This study identifies a NOAEL of 92 mg/kg/day for histopathological changes.

- *NCI (1978)* (Appendix, Tab 3) - Groups of 50 male and 50 female mice (B6C3F1) were exposed to each of two dose levels via corn oil gavage, 5 days/week for 78 weeks. Two control groups (vehicle and untreated) of 20 animals/sex were also included in the study. Initial doses of 0, 150, and 300 mg/kg-day were raised to 0, 200, and 400 mg/kg-day after week 20 since the animals seemed to tolerate exposure. Time-weighted average doses of 0, 195, and 390 mg/kg-day were calculated by the study authors. Mortality was significantly increased in exposed female mice, although this may not have been dose-related (mortality was greater in the low dose group compared to the high dose group). In any event, the number of animals surviving the entire

exposure exceeded the requirement of the OPPTS guideline for number of animals/dose group (10/sex/group). For male, there was no appreciable difference in mortality from controls. Body weight gain was not affected in either sex. Clinical signs (*i.e.*, appearance and behavior changes) were observed at comparable rates in exposed and control groups. Histopathological examinations revealed no significant differences between exposed and control animals for noncancer effects in any tissue site (including the respiratory tract). This study identifies a NOAEL of 390 mg/kg-day for noncancer histopathological effects in all tissues examined.

iii. Neurotoxicity

Oral data, summarized below and in the attached Table 2, provide a sufficient basis for EPA to assess the neurotoxicity of 1,1,2-trichloroethane:

- *White et al. (1985)* (Appendix, Tab 2) - Seven groups of male and female CD-1 mice were administered a single dose of 200 to 600 mg/kg 1,1,2-trichloroethane via gavage. Sedation and loss of righting reflex were noted in mice soon after receiving a single gavage dose of 450 mg/kg 1,1,2-trichloroethane or more. These animals recovered four hours after exposure. No gross changes to the central nervous system were noted upon necropsy. This study identifies an acute LOAEL of 450 mg/kg for the anesthetic effects of 1,1,2-trichloroethane.

- *NCI (1978)* (Appendix, Tab 3) - Groups of 50 male and 50 female rats (Osborne-Mendel) were exposed to each of two dose levels via corn oil gavage, 5 days/week for 78 weeks. Two control groups (vehicle and untreated) of 20 animals/sex were also included in the study. Initial doses of 0, 30, and 70 mg/kg-day were raised to 0, 50, and 100 mg/kg-day after week 8 since animals seemed to tolerate exposure. Time-weighted average doses of 0, 46, and 92 mg/kg-day were calculated by the study authors. During the first 6 months, the incidence of clinical signs (*i.e.*, appearance and behavior changes) was comparable between exposed and control animals. However, after 6 months, exposed animals exhibited a higher frequency of hunched appearance, rough fur, urine stains, dyspnea, and squinted eye (sometimes with reddish exudate). Histopathological examinations revealed no significant differences between exposed and control animals for noncancer effects in the brain or nerves. This study identifies a NOAEL of 92 mg/kg-day for histopathological effects on the central nervous system.

- *NCI (1978)* (Appendix, Tab 3) - Groups of 50 male and 50 female mice (B6C3F1) were exposed to each of two dose levels via corn oil gavage, 5 days/week for 78 weeks. Two control groups (vehicle and untreated) of 20

animals/sex were also included in the study. Initial doses of 0, 150, and 300 mg/kg-day were raised to 0, 200, and 400 mg/kg-day after week 20 since the animals seemed to tolerate exposure. Time-weighted average doses of 0, 195, and 390 mg/kg-day were calculated by the study authors. Clinical signs (*i.e.*, appearance and behavior changes) were observed at comparable rates in exposed and control groups. Histopathological examinations revealed no significant differences between exposed and control animals for noncancer effects in the brain and nerves. This study identifies a NOAEL of 390 mg/kg-day for histopathological changes.

iv. Developmental and Reproductive Toxicity

Testing for developmental and reproductive toxicity of 1,1,2-trichloroethane is unnecessary. A review of the available data quickly reveals that the likelihood of developmental and reproductive toxicity is negligible.

EPA posits a data gap for the developmental and reproductive toxicity of 1,1,2-trichloroethane, based on the availability of only a single study. That developmental study, by Seidenberg *et al.*, exposed mice to 1,1,2-trichloroethane during gestation. While significant toxicity was observed in the mice, no effects were observed in the pups to which they gave birth.[18] Although this study suffers from certain limitations (single dose tested, short exposure duration), it demonstrates a lack of developmental effects for 1,1,2-trichloroethane at a dose approximate to the maximum tolerable dose. Little information would be obtained from additional study since the testing of higher doses would only result in greater maternal toxicity, while the testing of lower doses would only confirm the absence of developmental effects at lower levels.

The weight of the available evidence on the developmental and reproductive effects of 1,1,2-trichloroethane, the potential for exposure, and the current regulatory structure all support the conclusion that the testing proposed is not necessary.

Although there is little information available regarding the potential for developmental effects of 1,1,2-trichloroethane, the data suggest that developmental effects are not of primary concern for chlorinated ethanes in general. Most studies have reported either no

[18] Groups of 30 ICR/SIM mice were exposed to 0 (vehicle control) or 350 mg/kg-day 1,1,2-trichloroethane on days 8 through 12 of gestation via corn oil gavage. Significant toxicity (3 deaths) was observed in exposed animals. However, no effects were observed on pup viability, pup weight, litter size, or terata. The dose level of 350 mg/kg-day serves as a LOAEL for material toxicity and a NOAEL for developmental effects. Seidenberg, *et al., Teratogenesis, Carcinogenesis, and Mutagenesis*, 6:361-374 (1986) (Appendix, Tab 4).

developmental effects or effects only at very high concentrations of chlorinated ethanes.[19] Therefore, when the negative results of the Seidenberg study are considered along with the weight of the negative evidence for structurally related compounds, additional study on the developmental effects of 1,1,2-trichloroethane does not appear to be necessary, particularly where potential human exposures are minimal and will remain minimal due to the potential carcinogenic risk.

Studies in which reproductive tissues were histopathologically evaluated have generally reported negative results. For example, in the NCI bioassay, no histopathological effects were observed in the testes, prostate, tunica vaginalis, uterus, mammary gland, and ovary in rats exposed orally to 46-92 mg/kg-day or in mice exposed orally to 195-390 mg/kg-day for 78 weeks.[20]

Available data also suggest that, as is the case with developmental effects, reproductive effects are not of primary concern for chlorinated ethanes in general. In this case as well, most studies have reported either no reproductive effects or effects only at very high concentrations of chlorinated ethanes.[21] In light of 1,1,2-trichloroethane's lack of histopathological effects on reproductive tissues, and the negative available data on structurally related compounds and very low human exposure potential, additional study on the reproductive effects of 1,1,2-trichloroethane does not appear to be necessary.

v. Carcinogenicity

There are adequate bioassay data, described below and in the attached Table 3, to assess the carcinogenicity of 1,1,2-trichloroethane:

- *NCI (1978)* (Appendix, Tab 3) - Groups of 50 male and 50 female rats (Osborne-Mendel) were exposed to each of two dose levels via corn oil gavage, 5 days/week for 78 weeks. Two control groups (vehicle and untreated) of 20 animals/sex were also included in the study. Initial doses of 0, 30, and 70 mg/kg-day were raised to 0,50, and 100 mg/kg-day after 8 days of exposure. Time-weighted average doses of 0, 46, and 92 mg/kg-day were

[19] See Appendix, Tab 5 for a table summarizing the developmental toxicity data for chlorinated ethanes.

[20] National Cancer Institute, *Bioassay of 1,1,2-Trichloroethane for Possible Carcinogenicity,* ISS DHEW/PUB/NIH-78-1324 (1978) (Appendix, Tab 3).

[21] See Appendix, Tab 6 for a table summarizing the reproductive toxicity data for chlorinated ethanes.

calculated by the study authors. High mortality was noted in the vehicle control group. Animals were followed up to 34 weeks after exposure. No statistically significant increase in tumor incidence was observed in exposed rats. The authors concluded that there was no convincing evidence for the carcinogenicity of 1,1,2-trichloroethane in rats. However, the maximum tolerable dose may not have been achieved in this study.

- *NCI (1978)* (Appendix, Tab 3) - Groups of 50 male and 50 female mice (B6C3F1) were exposed to each of two dose levels via corn oil gavage, 5 days/week for 78 weeks. Two control groups (vehicle and untreated) of 20 animals/sex were also included in the study. Initial doses of 0, 150, and 300 mg/kg-day were raised to 0, 200, and 400 mg/kg-day after week 20 of exposure. Time-weighted average doses of 0, 195, and 390 mg/kg-day were calculated by the study authors. Animals were followed for up to 12 weeks following exposure. Mortality was significantly increased in exposed female mice, however this may not have been dose-related (mortality was greater in the low dose group compared to the high dose group). The incidence of hepatocellular carcinomas was significantly increased in exposed mice of both sexes. Additionally, adrenal pheochomocytomas were elevated in female mice. The authors concluded that 1,1,2-trichloroethane was carcinogenic to mice under the conditions of this bioassay.

EPA has used these data to develop both oral and inhalation cancer potency factors on the IRIS data base (Appendix, Tab 7). A repetition of the cancer bioassay for 1,1,2-trichloroethane would not be a justifiable use of laboratory animals, particularly in light of the fact that a chronic bioassay (by subcontoneous injection) is also available (Norpoth *et al.* 1988).[22]

vi. Immunotoxicity

Adequate data are available to assess the immunotoxicity of 1,1,2-trichloroethane, as described below and in the attached Table 4:

- *Sanders et al. (1985)* (Appendix, Tab 8) - Groups of 32-48 male and 32-48 female CD-1 mice were exposed to doses of 0, 4.4, 46, or 305 mg/kg-day (males), or 0, 3.9, 44, or 384 (females) 1,1,2-trichloroethane in the drinking water for 90 days. Drinking water consumption and body weight gain were reduced in concentration-dependent manner in male mice, but not in female mice. Spleen weights were decreased in a dose-dependent manner in

[22] Norpoth, K. *et al.*, Investigations on the Metabolism and Carcinogenicity of 1,1,2-Trichloroethane, *J. Cancer Res. Clin. Oncol.* 114: 158-162 (1988).

male mice, but were significantly increased in females exposed to the highest dose. Bone marrow status as indicated by DNA synthesis was not affected by exposure. Cell-mediated immunity, as measured by delayed-type hypersensitivity and popliteal lymph node proliferation responses to sheep erythrocytes, was unaffected in both sexes. However, humoral immune status was significantly depressed in both sexes in a dose dependent manner. For example, hemagglutination titers were significantly depressed in both sexes at the two highest doses. In addition, lymphocyte responsiveness to Con A and LPS was significantly depressed in female mice exposed to the highest dose, but unaffected in exposed male mice. This study identifies a LOAEL of 44-46 mg/kg-day and a NOAEL of 3.9-4.4 mg/kg-day for depression of humoral immunity.

- *NCI (1978)* (Appendix, Tab 3) - Groups of 50 male and 50 female mice (B6C3F1) were exposed to each of two dose levels via corn oil gavage, 5 days/week for 78 weeks. Two control groups (vehicle and untreated) of 20 animals/sex were also included in the study. Initial doses of 0, 150, and 300 mg/kg-day were raised to 0, 200, and 400 mg/kg-day after week 20 since the animals seemed to tolerate exposure. Time-weighted average doses of 0, 195, and 390 mg/kg-day were calculated by the study authors. Histopathological examinations revealed no significant differences between exposed and control animals for noncancer effects in immunological tissues (*i.e.*, spleen, bone marrow, lymph nodes). This study identifies a NOAEL of 390 mg/kg-day for histopathological effects.

vii. *In Vivo* Cytogenicity

Testing for *in vivo* cytogenicity is unnecessary for reasons directly opposite those described above for acute toxicity. Existing studies have already identified a genotoxic effect for 1,1,2-trichloroethane *in vivo*, as well as a cytogenetic effect *in vitro*. One can reasonably assume that genotoxic and cytogenetic effects would also be observed following inhalation exposures to 1,1,2-trichloroethane.

2. EDC

The available information on EDC, like that for 1,1,2-trichloroethane, is sufficient for screening analysis and residual risk determinations. EPA has proposed inhalation testing in the following areas: acute toxicity, subchronic toxicity, developmental toxicity, reproductive toxicity, and neurotoxicity. The estimated cost of these tests is approximately $2.4 million. A review of the data currently available on EDC indicates that additional testing is not necessary

for these endpoints. Oral studies for acute toxicity, subchronic toxicity, reproductive toxicity, and neurotoxicity are already available. These studies can serve as the basis for route-to-route extrapolation to gauge inhalation effects and fill any data gaps that may concern EPA. As for developmental toxicity, an inhalation study that appeared after EPA had completed its literature search for the proposal provides the data that the Agency sought in that area.[23] Indeed, in response to the PK proposal submitted by the HAP Task Force, EPA has expressly recognized that this study adequately addresses the Agency's data need in the area of developmental toxicity testing.[24]

i. Acute Toxicity

There is an adequate study available to assess the acute effects of EDC, as described below and in the attached Table 5:

- *Daniel et al. (1994)* (Appendix, Tab 11) - Groups of 10 male and 10 female Sprague-Dawley rats were exposed to 0, 10, 30, 100, or 300 mg/kg-day EDC via corn oil gavage for 10 days. Significant mortality (10/10 females, 8/10 males) was noted at the highest dose. Body weight, clinical chemistry, and hematological findings in exposed animals were not significantly different from controls. The main histopathological change noted was inflammation of the forestomach in animals receiving 100 mg/kg-day or more. This endpoint represents a direct contact effect of EDC, which is attributable in part to the mode of administration (*i.e.,* high concentrations administered as a bolus dose in corn oil). Since this effect is not extrapolatable to inhalation exposures, this study identifies a NOAEL of 100 mg/kg-day for histopathological changes in the liver and kidney.

It is unlikely that EDC would have portal-of-entry effects. EDC does not accumulate preferentially in the lungs following inhalation exposure, but distributes rapidly to other tissues. Following inhalation exposure to 50-250 ppm EDC for two to three hours, the levels of EDC in the lungs were lower than those observed in the blood and much lower than those observed in adipose tissue.[25]

[23] Payan *et al.*, *Assessment of the Developmental Toxicity and Placental Transfer of 1,2 Dichloroethane in Rats*, Fund. Appl. Toxicol. 28: 189-198 (1995) (Appendix, Tab 9).

[24] Environmental Protection Agency, *Preliminary EPA Technical Analysis of Proposed Industry Pharmacokinetics (PK) Strategy for Ethylene Dichloride (EDC)* (June 1997), p. 9 (Appendix, Tab 10).

[25] Speafico F. *et al.*, Pharmacokinetics of Ethylene Dichloride in Rats Treated by Different Routes and its Long-Term Inhalatory Toxicity. In: Ames B.N. *et al.*, Ethylene Dichloride: A Potential Health Risk?, Banbury

ii. Subchronic Toxicity

There are a number of subchronic studies of EDC by the oral route, which are certainly adequate to assess its subchronic toxicity. These are summarized below and in the atached Table 6:

- *NTP (1991 - Drinking Water Study in Rats)* (Appendix, Tab 12) - Groups of 10-20 male and 10 female F344/N, Osborne-Mendel, and Sprague-Dawley rats were exposed to drinking water containing 0, 500, 1,000, 2,000, 4,000, or 8,000 ppm EDC for 13 weeks. The actual doses received by the animals varied slightly between species and sex, but were generally between 0, 49-82, 86-126, 145-213, 259-428, 515-727 mg/kg-day for the respective water concentrations. Mortality was not significantly affected by exposure in any strain or sex. This study identifies a NOAEL and LOAEL of 58 and 102 mg/kg-day, respectively, for the effects of EDC on the kidney in F344/N rats. The remaining findings from this study are discussed according to rat strain below:

  F344/N - Water intake and body weights were affected in F344/N rats at the two highest doses. No compound-related clinical signs were noted. The authors attributed slight changes in hematological parameters to mild dehydration. Liver and kidney weights were elevated in all exposed animals compared to controls. Histopathological changes in the liver were not observed. While mild renal tubular regeneration was comparable to controls in exposed male rats, the incidence of this effect was increased in a dose-dependent manner in female rats exposed to 1,000 ppm or more.

  Sprague-Dawley - Water intake and body weights were affected in Sprague-Dawley rats at the two highest doses. No compound-related clinical signs were noted. The authors attributed slight changes in hematological parameters to mild dehydration. Liver and kidney weights were elevated in all exposed animals compared to controls. Histopathological changes in the liver were not observed. The incidence of mild renal tubular regeneration was comparable to controls in exposed male and female rats.

  Osborne-Mendel - Water intake and body weights were affected in Osborne-Mendel rats exposed to 1,000 ppm (females) and 2,000 ppm (males) or more. No compound-related clinical signs were noted. The authors attributed slight changes in hematological parameters to mild dehydration. Kidney weights were elevated in all dosed females.

Report No. 5 (Cold Spring Harbor, New York), pp. 107-133.

Liver weights were elevated in exposed males receiving 1,000-2,000 ppm. Histopathological changes in the liver were not observed. The incidence of mild renal tubular regeneration was not significantly different from controls in exposed male and female rats.

- *NTP (1991 - Corn Oil Gavage Study in Rats)* (Appendix, Tab 12) - Groups of 10-20 male and 10 female F344/N rats were exposed to 1, 30, 60, 120, 240, or 480 mg/kg-day (males) or 1, 18, 37, 75, 150, or 300 mg/kg-day (females) EDC via corn oil gavage for 13 weeks. Significant mortality was noted in male rats (all) receiving 240 mg/kg-day or more, and in female rats (9/10) exposed to 300 mg/kg-day. Body weights were decreased in rats exposed to the highest dose. Liver and kidney weights were elevated in all exposed animals, however histopathological changes in these tissues were not observed. Forestomach effects (hyperplasia, inflammation, and mineralization) were noted in animals that died or were moribund. Necrosis of the thymus was observed in males exposed to 240 mg/kg or more, and in females exposed to 300 mg/kg. The incidence of renal tubular regeneration was comparable between exposed and control animals. Histopathological changes in the liver were not observed. Neurological effects were also observed in animals exposed to the highest doses of EDC. This study identifies a LOAEL of 120 mg/kg-day for necrosis of the thymus.

- *NTP (1991 - Drinking Water Study in Mice)* (Appendix, Tab 12) - Groups of 10 male and 10 female B6C3F1 mice were exposed to drinking water containing 0, 500, 1,000, 2,000, 4,000, or 8,000 ppm EDC for 13 weeks. These concentrations corresponded to doses of 1, 249, 448, 781, 2,710, or 4,207 mg/kg-day (males) and 0, 244, 647, 1,182, 2,478, or 4,926 mg/kg-day (females). Significant mortality (9/10) limits the interpretation of results from female mice exposed to the highest dose. All other exposed animals survived the full 13 week exposure. Compound-related clinical signs were not observed in any dose group. Body weights were lower in all exposed males, and in females exposed to 1,000 ppm or more. Liver and kidney weights were elevated in all exposed animals. In addition, mild-to-moderate tubular regeneration was noted in males exposed to the two highest doses. Histopathological changes in the liver were not observed. This study identifies a NOAEL and LOAEL of 781 and 2,710 mg/kg-day for renal effects in mice.

- *Daniel et al. (1994)* (Appendix, Tab 11) - Groups of 10 male and 10 female Sprague-Dawley rats were exposed to 0, 37.5, 75, and 150 mg/kg-day EDC via corn oil gavage for 90 days. No treatment-related effects were noted regarding mortality, clinical observations, ophthalmology, gross pathology, or histopathology in exposed animals. Body weight gain and food consumption were significantly decreased in male rats exposed to the highest dose.

Statistically significant differences in hemoglobin, hematocrit, red blood cell count, platelets, albumin, and alkaline phosphatase were noted in animals exposed to 75 mg/kg-day or more. Organ weight changes (liver, kidney, brain) were also noted in animals exposed to the two highest doses. This study identifies a LOAEL of 75 mg/kg-day and NOAEL of 37.5 mg/kg-day for hematological effects and organ weight changes.

iii. Reproductive Toxicity

Three reproductive studies are currently available for EDC (Lane *et al.* 1982; Rao *et al.* 1980; Alumot *et al.* 1976). They are described briefly below:

- *Lane et al. (1982)* (Appendix, Tab 13) - In a multigeneration study, groups of 10 male and 30 female ICR Swiss mice were exposed to 1, 5, 15, or 50 mg/kg-day EDC via the drinking water. The F0 generation was exposed for 5 weeks prior to mating, whereas the F1 generation was exposed for 11 weeks prior to mating. No treatment-related effects on fertility, gestation, terata, pup weight gain, pup survival, or dominant lethal mutations were observed. This study identifies a NOAEL of 50 mg/kg-day for the reproductive effects of EDC.

- *Rao et al. (1980, also cited as Murray et al. 1980, Schlacter et al. 1979, and Shell Oil 1979)* (Appendix, Tab 14) - In a single generation study, groups of 20-30 male and female Sprague-Dawley rats were exposed via inhalation to 0, 25, 75, or 150 ppm EDC for 6 hours/day beginning 60 days prior to mating, and continuing through gestation (F1a and F1b). No treatment related effects were observed on fertility index, pup survival, gestation length, sex ratio, or organ weights in pups from either the F1a or F1b litters. This study identifies a NOAEL of 150 ppm for the reproductive effects of EDC.

- *Alumot et al. (1976)* (Appendix, Tab 15) - In a single generation study, groups of 18 male and 18 female rats (species not specified) were exposed to mash fumigated with EDC. Residue levels measured 250 and 500 ppm, corresponding to doses of approximately 13 and 25 mg/kg-day, respectively. After 6-weeks on the test diet, females were mated with untreated males. Thereafter, at 2-monthly intervals, 45 treated males were mated with treated females for a total of 24 months. Following mating, females were weighed twice weekly. Litter size and weight were recorded at parturition and at 10 days. Liver fat content and serum biochemistry analyzes were performed on parental animals at the end of the treatment period, and did not reveal significant effects. No treatment related effects were reported on reproductive success in animals followed for up to 5 sequential pregnancies during a two-year exposure period. This study provides excellent information

regarding the potential for reproductive effects in aging animals (an important endpoint which is *not* specifically addressed in the TSCA testing requirements). This study identifies a NOAEL of 25 mg/kg-day for the reproductive effects of EDC.

Although neither Rao *et al.* (1976) nor Alumot *et al.* (1976) satisfy all current TSCA testing requirements, these studies do provide useful information regarding the potential for reproductive effects of EDC. Because a second generation was evaluated by Lane *et al.* (1982), it warrants additional consideration. The study design for Lane *et al.* (1982) was approved by EPA. A comparison of this study to current TSCA testing requirements is provided below:

Points where requirements are met by Lane *et al.* (1982)

- Adequacy of test species: Swiss mice
- Adequate number of dose groups: Four (including control)
- Adequate study design: Two generations
- Adequate evaluation of the following endpoints: gross necropsy, body weight, fertility index, gestation/lactation index.

Points where requirements are exceeded by Lane *et al.* (1982)

- Number of pregnant animals/dose group: 20 required, 30 tested
- Number of F1 and F2 matings: 1 F1 required, 3 tested; 1 F2 required, 2 tested
- Dominant lethal mutations: Not required under TSCA

Points where requirements are not met by Lane *et al.* (1982)

- Lack of evaluation of the following endpoints: organ weights, histopathology, estrous cyclicity, sperm count/morphology, age at sexual maturation.

The potential limitations of Lane *et al.* (1982) are discussed below:

- *Organ Weights* - Organ weights were not measured in F0, F1, or F2 animals by Lane *et al.* (1982). However, this endpoint is of questionable significance, since changes in organ weight usually do not constitute an adverse effect. Furthermore, since organ weights for reproductive tissues were reported in several other studies without remarkable findings (Cheever *et al.* 1990; Daniel *et al.* 1994; Rao *et al.* 1980; NTP, 1991), this does not constitute a serious limitation in the study design.

- *Histopathology* - Histopathological examinations of F0, F1, or F2 animals were not conducted by Lane *et al.* (1982). Regarding F0 animals, several other studies have evaluated histopathology of reproductive tissues following oral and inhalation exposures (Rao *et al.* 1980; NTP, 1991; Daniel *et al.* 1994; Cheever *et al.* 1990), none of which report any histopathological change in reproductive tissues. In addition, histopathological changes of the liver, kidney, and any other target organs noted during gross examination have been evaluated in F1 rat pups (Rao *et al.* 1980), again without notable effects. However, regarding F1/F2 animals, this is no longer required:

> "EPA dropped the requirement of histopathology of developmental anomalies observed macroscopically in F1 and F2 weanlings."

63 Fed. Reg. 43820, 43822 (Aug. 15, 1997). For this reason, the absence of histopathology does not constitute a significant limitation in this study.

- *Sperm Count, Motility, Morphology* - Sperm structure and function were not evaluated by Lane *et al.* (1982). The dominant lethal mutation assay (which was conducted by Lane *et al.*) is, however, a more relevant endpoint for EDC, since it is recognized as a DNA-reactive chemical.

- *Estrous Cyclicity* - Estrous cyclicity was not evaluated by Lane *et al.* (1982). Halogenated organic compounds that are known to affect the estrous cycle (such as DDT, methoxychlor, PCBs) are generally believed to produce their effects via interaction with the estrogen receptor. Given the chemical structure of EDC, it is very unlikely that it exerts any estrogenic (or other hormonal) activity.

- *Age at Sexual Maturation* - The age at sexual maturity (day of vaginal opening/preputial separation) was not assessed by Lane *et al.* (1982). Like estrous cyclicity, halogenated organic chemicals which are known to speed or delay the onset of sexual maturity (including DDT, methoxychlor, polychlorinated biphenyls, polybrominated biphenyls) are generally believed to produce their effects via interaction with the estrogen receptor. Given the chemical structure of EDC, it is very unlikely that it exerts any estrogenic (or other hormonal) activity.

In summary, the deficiencies noted for Lane *et al.* (1982) can be characterized as minor. Two endpoints (organ weight, histopathology) are readily addressed by other studies, or are no longer applicable. The remaining three endpoints (sperm morphology, estrous cyclicity, age at sexual maturation) are more appropriately characterized as those aimed at

understanding the mechanism by which a chemical affects reproductive success. Because the weight of evidence (Alumot *et al.* 1976; Rao *et al.* 1980; Lane *et al.* 1982) clearly indicates that EDC has no effect on reproductive success, the importance of these mechanistic endpoints is greatly reduced. Furthermore, the likelihood of obtaining a positive response for these endpoints (if assayed) is small. Additional testing for the reproductive effects of EDC is not warranted.

iv. Neurotoxicity

Adequate studies to evaluate the neurotoxic potential of EDC are described below and in the attached Table 7:

- *NTP (1991 - Drinking Water Study)* (Appendix, Tab 12) - Groups of 10-20 male and 10 female F344/N, Osborne-Mendel and Sprague-Dawley rats, and 10 male and 10 female B6C3F1 mice were exposed to drinking water containing 0, 500, 1,000, 2000, 4,000, or 8,000 ppm EDC for 13 weeks. Significant mortality (9/10) limits the interpretation of results from female mice exposed to the highest dose. All other exposed animals survived the full 13 week exposure. No compound-related clinical signs, changes in brain weight, or histological changes of the central nervous system (brain and spinal cord) were observed in any of the exposed animals. Although no LOAELs were identified, this study identifies a NOAEL of 8,000 ppm for EDC (approximately 492 mg/kg-day in rats and 4,207 mg/kg-day in mice) for neurological effects.

- *NTP (1991 - Corn Oil Gavage Study in Rats)* (Appendix, Tab 12) - Groups of 10-20 male and 10 female F344/N rats were exposed to 0, 30, 60, 120, 240, or 480 mg/kg-day (males or 0, 18, 37, 75, 150, or 300 mg/kg-day (females) EDC via corn oil gavage for 13 weeks. Significant mortality was noted in male rats (all) receiving 240 mg/kg-day or more, and in female rats (9/10) exposed to 300 mg/kg-day. Clinical signs, including tremors, salivation, emaciation, abnormal posture, ruffled fur, and dysnea were noted in males exposed to 240 mg/kg-day and in females exposed to 300 mg/kg-day. In addition, necrosis of the cerebellum was observed in males exposed to 240 mg/kg or more, and in females exposed to 300 mg/kg. This study identifies a LOAEL of 240 mg/kg-day and a NOAEL of 120 mg/kg-day for clinical signs of neurotoxicity and necrosis of the cerebellum.

3. VDC

As in the case of 1,1,2-trichloroethane, the low exposure profile of VDC enables EPA to base residual risk determinations on existing data, which are more than adequate for screening and residual risk assessments. EPA proposes to require acute and neurotoxicity tests by the inhalation route for VDC, at a cost of over $500,000. Yet, as described in section II above, the number of workers potentially exposed to VDC is well below the 1000-worker threshold. Moreover, as described more fully in section II, there is no basis in the record from which to conclude that the general population or consumer thresholds are met. Ambient air levels are extremely low.

Acute exposure of humans and animals to high concentrations of VDC results in CNS depression and unconsciousness, which are consistent with observations for many organic solvents. There have been no known reported deaths resulting from acute accidental exposures to VDC, and individuals that have been acutely exposed have completely recovered.[26] No histopathological effects were observed in lung tissue related to VDC exposure at 100 ppm for 6 weeks in dogs, guinea pigs, monkeys, rabbits, or rats.[27] In other studies feeding dogs 25 mg VDC/kg-day for 97 days or rats 30 mg VDC/kg-day for 2 years did not cause any adverse neurological effects.[28] Furthermore, the available toxicokinetic data do not indicate a route-specific target organ specificity for VDC. Based on the existing data, additional acute testing and intermediate dosing experiments for neurological effects do not appear to be warranted.

C. EPA Failed To Consider the Impact of MACT Standards on Exposure

In addition to failing to consider the physical and chemical properties of the HAPs and other chemical-specific data, EPA did not take into account the changing conditions under

[26] EPA, *Status Assessment of Toxic Chemicals: Vinylidene Chloride* (1979).

[27] Prendergast J.A. *et al., Effects on Experimental Animals of Long-term Inhalation of Trichloroethylene, Carbon Tetrachloride, 1,1,1-Trichloroethane, Dichlorodifluoromethane, and 1,1-Dichloroethylene*, Toxicol. Appl. Pharmacol. 10: 270-289 (1967).

[28] Quast J.F. *et al., A Chronic Toxicity and Oncogenicity Study in Rats and Subchronic Toxicity Study in Dogs on Ingested Vinylidene Chloride*, Fundam. Appl. Toxicol. 3(1): 55-62 (1983).

which they are emitted. In particular, EPA has not considered the impact of the adoption of MACT standards for a number of source categories, which will dramatically restrict the emissions of HAPs. Section 112(d) of the Clean Air Act directs EPA to set national emissions standards for HAPs (NESHAPs) which shall require:

> the maximum degree of reduction in emissions of the hazardous air pollutants subject to this section (including a prohibition on such emissions, where achievable) that the Administrator, taking into consideration the cost of achieving such emission reduction, and any non-air quality health and environmental impacts and energy requirements, determines is achievable for new and existing sources in the category or subcategory to which such emission standard applies.

This maximum degree of reduction in emissions is subject to a "floor," *i.e.*, a minimum level of reduction that must be achieved. For new sources, the emissions standards shall not be set any lower than "the emission control that is achieved in practice by the best controlled similar source." For existing sources, the standards must be no less stringent than the average emission limitation that is achieved by the best performing 12 percent of existing sources in each category or subcategory of 30 or more sources (for smaller categories or subcategories, the average of the best performing five sources is used).

Pursuant to Section 112(d), EPA promulgated a rule setting a hazardous organics NESHAP regulating emissions of listed hazardous air pollutants from the synthetic organic chemical manufacturing industry (SOCMI). The rule imposed MACT standards on all major SOCMI sources. Among the chemicals covered by the hazardous organics NESHAP are EDC, VDC, and 1,1,2-trichloroethane. They are subject to the MACT restrictions, which EPA itself estimates will significantly reduce emissions from the SOCMI industry, perhaps by as much as 88 percent.[29] There is no recognition of this point in the proposed test rule. The 1993 TRI data upon which EPA based its decisionmaking for the HAP test rule do not reflect the reductions that compliance with the hazardous organics NESHAP will bring about. Yet the Agency does not consider whether the ongoing reductions pursuant to this and other MACT standards will diminish the risk to health and the environment sufficiently to curtail the need for expensive testing. Nor, as discussed below, does it consider whether in some cases

[29] 59 Fed. Reg. 19402, 19411 (April 22, 1994).

the reductions will result in no major sources of certain HAPs remaining in existence and hence no statutory basis for the test rule for such HAPs.

The preamble to the proposed test rule does not indicate that there has been any inquiry into the actual current level of emissions of the HAPs, or whether the MACT standards are achieving the restrictions that they were intended to bring about. The Agency does not even appear to have considered whether the "floor" standards for new and existing sources affect the need for testing. There is no discussion of what emission control has been "achieved in practice by the best controlled similar source" or what the average emissions limitation achieved by the best performing 12 percent of existing sources has been. These issues pertain not only to the need for testing, but also to the uses to which EPA will put the information that it obtains. The Agency justifies the test rule on the basis that it will use the information to conduct residual risk evaluations under the Clean Air Act. If that is the case, since the statute requires residual risk evaluations to be made after MACT is in place, the test rule should be based on projected emissions and exposure under MACT. By disregarding this aspect of the statutory requirements, EPA undermines its own goals.

III. EPA SHOULD PROVIDE FOR TIERED TESTING, RATHER THAN APPLYING OPTION THREE ACROSS THE BOARD

In considering how broad and how deep a data set to require on each HAP, EPA listed four options that it had reviewed as the range of possibilities. Briefly, these options are:

- Option 1: one-species 90-day inhalation subchronic plus follow-up for known or suspect toxicities;
- Option 2: option 1 plus inhalation screening for untested toxicity endpoints;
- Option 3: option 1 plus less than chronic testing for noncancer endpoints of concern;
- Option 4: Option 1 plus chronic testing.

The baseline for each of these considerations is Option 1 testing, which consists of a one-species 90-day inhalation subchronic test, as well as testing for endpoints that have already been identified as existing or potential concerns by previous test results for the HAP at issue

or structurally similar agents. EPA would augment these studies by testing for adverse health effects that are suggested or indicated, but not adequately characterized, by existing information such as short-term test data, mechanistic information, or structure-activity relationships.

Of these choices, EPA selected Option 3. This would mandate not only the Option 1 90-day subchronic inhalation studies, but also inhalation testing to assess reproductive effects (that is, a two-generation reproductive toxicity study), as well as developmental toxicity tests in two mammalian species. Option 3 also includes an acute toxicity testing guideline for histopathology of the respiratory tract, the kidney, and the liver, as well as a broncheoalveolar lavage after four hours of exposure.[30] This is a wide array of endpoints, testing for which will be expensive and time-consuming. Moreover, not every one of these endpoints will be appropriate to each HAP. The HAPs are diverse in terms of their levels of emissions or exposure and ambient air concentrations. Their physical or chemical properties, *e.g.*, their known or suspected toxicity and the pathways that they will take through the body, vary widely. By selecting Option 3, EPA fails to take these factors into account, and seeks to paint with a broad, one-size-fits-all brush.

In light of the assortment of chemicals encompassed by the proposed test rule, Option 2 is a more appropriate selection. Option 2 also begins with the Option 1 baseline of tests, but then adds a more open-ended set of supplemental studies. It would require a one-generation screening test for reproductive effects, a subchronic inhalation neurotoxicity screening battery, an *E. coli* reverse mutation assay, gene mutation in somatic cells in culture detection, an *in vivo* cytogenetics test, and an immunotoxicity screening test. Any toxicity suggested but not characterized by existing studies in the toxicological literature would be followed up under more rigorous protocols. This is a more flexible approach, with an emphasis on screening studies to determine the level and likelihood of a particular risk for each individual HAP prior to ordering more in-depth investigations. Such a course enables EPA to tailor test requirements to the characteristics of each HAP, thus conserving both agency and industry resources.

[30] 61 Fed. Reg. at 33182.

EPA rejected Option 2, even though it conceded that it "would conserve resources while allowing for the testing of a broader range of endpoints, including cancer." The only reason it offered was that it considered a one-generation reproductive test inadequate, and thought that additional follow-up testing would be required to confirm suggestive results from the screening studies, which would necessitate a second round of rulemaking. Such an inflexible approach should not preclude the use of a more efficient testing scheme. The purpose of the screening tests would be to identify those HAPs that pose a risk of reproductive toxicity and which warrant further studies, while winnowing out those that do not, thus relieving manufacturers of the need for unnecessary testing. Criteria can easily be developed to determine when a screening test merits further investigation, and additional screening methods identified to buttress the reliability of the initial test.

EPA's general opposition to tiered testing will not withstand scrutiny. EPA suggests that such testing would be time-consuming, require multiple rulemakings, and be a burden on EPA resources. To the contrary, an approach carefully designed at the outset to develop data that will be useful for residual risk determinations will conserve time and Agency resources, not to mention avoiding the needless killing of thousands of test animals. EPA also justifies Option 3 on the grounds that it has prioritized HAPs based on exposure potential. It is clear, however, that EPA has looked only at releases, and has neglected to assess exposure potential.

Most significantly, EPA asserts a need for a consistent even data base for the HAPs across a broad range of endpoints. To conduct the residual risk analyses required under Section 112(f) of the Clean Air Act, however, EPA only needs sufficient data to characterize the health effects of an HAP at reasonably expected exposure levels. In some cases, this might be a single chronic study supporting regulation at very low levels. EPA lacks statutory support for going further in such cases and developing a "uniform" data base of test results that can be seen at the outset to be completely irrelevant to a determination of residual risk. EPA should follow the recommendation of the National Academy of Sciences that it adopt an iterative approach to develop test data for the HAPs.[31]

[31] National Academy of Sciences, *Science and Judgment in Risk Assessment* (1994), p. 84.

IV. EPA HAS NOT SUFFICIENTLY EXPLAINED THE PURPOSE OF THE TEST RULE

A. EPA Has Not Shown how Data Developed under the Test Rule Are Necessary for Residual Risk Determinations

1. EPA Has Not Prepared the Report on Residual Risk Determinations Required by Statute

EPA has stated that the primary purpose of the proposed test rule is to obtain data to support residual risk analysis under Section 112 of the Clean Air Act.[32] Although the Act was amended seven years ago to require such an analysis, EPA has yet to articulate how it will make residual risk determinations. Section 112(f) directs EPA to examine the impact of the MACT standards on emissions and to determine whether the residual risk to the public from non-cancer adverse health effects falls within an "ample margin of safety," and whether any excess risk of cancer is lower than one in a million. In conjunction with this evaluation, EPA must determine whether additional, post-MACT emissions limits are needed to protect public safety. The Act requires EPA to present a report to Congress by November 15, 1996 explaining how it would conduct this evaluation.

On April 22, 1998, EPA issued for public comment the draft report to Congress on residual risk. 63 Fed. Reg 19914. Although the draft report is 129 pages in length, nowhere does it explain how data developed under the HAP test rule as proposed will materially assist EPA in making residual risk determinations for animal carcinogens. The draft report speaks entirely in generalities and uses terminology and flow charts that seem designed to obscure the process. It studiously avoids these important questions: How will EPA use the results of expensive studies involving thousands of test animals in evaluating whether to lower exposures to HAPs that it already regulates on the basis that there is no safe threshold because of their potential carcinogenicity? What purpose is served by obtaining additional test data for such compounds? Can EPA point to any carcinogen for which non-cancer test data has driven regulations to a lower level?

With no such explanations available, neither EPA nor the public can judge what data will be necessary for the residual risk evaluation, or what kind of testing will be appropriate to

[32] 61 Fed. Reg. at 33179.

gather it. The exchange of information and ideas normally associated with the notice and comment process are hamstrung by this limitation. EPA should not propose such broad and onerous testing requirements until it has completed its policy for residual risk determinations and identified the type of information that it will utilize in making them.

2. The Proposed Test Rule Does Not Serve the Goals Identified by the Committee on Risk Assessment and Risk Management for Residual Risk Determinations

While EPA has not completed its report on risk assessment, the Congressionally established Commission on Risk Assessment and Risk Management has done so, and the recommendations in its *Framework for Environmental Health Risk Management* highlight the shortcomings of the test rule proposed by EPA.

The Commission recommends that EPA utilize a tiered scheme, conducted with stakeholder involvement, consisting of the following steps: (i) characterize and articulate the scope of the national, regional, and local air toxics problems and their public health and environmental contexts; (ii) use available data and default assumptions to perform screening level risk assessments to identify sources with the highest apparent risks; (iii) conduct more detailed assessments of sources and facilities with the highest risks; (iv) at facilities with incremental lifetime upper-bound cancer risks greater than one in 100,000 persons exposed or that have exposure standards, examine and choose risk reduction options in light of total facility risks and public health context; and (v) consider reduction of residual risks from source categories of lesser priority.[33]

The Commission noted that EPA is not well prepared to implement such a tiered risk assessment because "[c]ritical information gaps exist that hinder EPA from reliably determining to what extent MACT standards are reducing health risks and whether significant residual risks remain." The proposed test rule will not fill these existing gaps. For example, the Commission notes that the analysis under the first step in the risk assessment scheme should put air toxics in the context of exposures from other air pollutant sources and other

[33] Commission on Risk Assessment and Risk Management, *Framework for Environmental Health Risk Management* (1997), p. 109 (Appendix, Tab 16).

environmental pathways. The second step, screening risk assessments, involves consideration of stack heights, distances to fence lines, emission rates, and "lookup tables" to estimate maximum offsite concentrations, as well as the size of the exposed populations. Yet, as noted above, the proposed test rule gives no consideration to exposure or exposed populations. The Commission concluded its section on Hazardous Air Pollutants by stating that "[b]y looking at hazardous air pollutants in the larger context of air pollution in particular geographic areas, EPA will be able to make more informed decisions about reducing residual emissions."[34] The proposed test rule fails to take such a course.

B. The Statute Does Not Require Residual Risk Analysis for VDC and 1,1,2-Trichloroethane, and Available Carcinogenicity Data Suffice for Such Determinations for those HAPs as Well as EDC

Section 112(f) of the Clean Air Act does not require residual risk determinations where there are no major sources of an HAP remaining after implementation of MACT standards. Only a handful of the facilities currently emitting VDC or 1,1,2-trichloroethane are major sources, and there will be even fewer, if any, as the hazardous organics NESHAP or other MACT standards are implemented. According to the 1995 TRI data, there are only *two* major sources of VDC emissions -- one of which only exceeded the 20,000 pounds a year threshold for emissions by 900 pounds -- and only *five* major sources of 1,1,2-trichloroethane emissions, three of which exceeded the 20,000 pound threshold by fewer than 3,000 pounds (Appendix, Tab 17). This reflects a significant reduction in number of major sources since the 1993 TRI report. Yet EPA would impose a test rule for these two substances with estimated test costs of over $500,000 and nearly $4 million, respectively. This is not an effective use of resources. If current trends continue, there may well be no major sources of either HAP after 2000.

In light of these figures, EPA will be hard-pressed under Executive Order 12866 -- which provides that federal agencies shall, in deciding whether and how to regulate, assess all costs and benefits of available regulatory alternatives, including the alternative of not regulating -- to justify the cost of testing to conduct residual risk determinations that may not even have to be done.

[34] *Id.* at 110.

Another cost ignored by EPA is the loss of animal life that this rule would entail. To conduct the tests required by the proposed test rule would mean the sacrifice of thousands of test animals, all to obtain data that EPA has not yet decided how to use. As an ethical matter, such a program should not be entered into lightly, without careful consideration of the need for the testing and the way that the test results will be used to benefit public health. EPA does not seem to have even acknowledged this issue as a factor in its decisionmaking.

While the costs of the proposed test rule are significant from both a financial and ethical standpoint, the potential benefits are meager. EDC, VDC, and 1,1,2-trichloroethane are already considered possible or probable human carcinogens by EPA, and it is not clear how acute, neurotoxicity, immunotoxicity, developmental, or reproductive toxicity studies would be of any use to a residual risk determination. IRIS lists a unit risk estimate of cancer potency for EDC of 2.6×10^{-5} ($\mu g/m^3$) (Appendix, Tab 18). The unit risk estimate for VDC is 5.0×10^{-5} ($\mu g/m^3$) (Appendix, Tab 19) and for 1,1,2-trichloroethane is 1.6×10^{-5} ($\mu g/m^3$) (Appendix, Tab 7). Accordingly, these HAPs are already subject to strict emissions or discharge limits where they are regulated as carcinogens. For example, the maximum contaminant level goal (MCLG) for EDC in drinking water is 0, and the maximum contaminant level is 5 $\mu g/l$. The MCLG for VDC is 7 $\mu g/l$, with an MCL of 7 $\mu g/l$. The MCLG for 1,1,2-trichloroethane is 3 $\mu g/l$ with an MCL of 3 $\mu g/l$.

It is inconceivable that EPA would make these requirements stricter as a result of acute, neurotoxicity, immunotoxicity, developmental, or reproductive toxicity tests. Carcinogens have traditionally prompted the imposition of the strictest possible standards by EPA, because of its application of a non-threshold model to estimate risk. The other effects, on the other hand, are generally recognized as threshold effects. Whatever the results of such testing for EDC, VDC, or 1,1,2-trichloroethane, regulation to reduce potential cancer risk to the most exposed individual to 1×10^{-6} or even 1×10^{-5} will ensure an "ample margin of safety" for the non-cancer effects.

C. The Alternative Justifications That EPA Provides For The Test Rule Also Do Not Support Its Breadth

It is not realistic to suggest that the Agency will ease the requirements or "de-list" any of these substances under Section 112 if the developmental and reproductive toxicity or other required test results are negative. Regardless of the outcome of those tests, EDC, VDC, and 1,1,2-trichloroethane will remain HAPs. Their rankings as possible or probable human carcinogens will not be affected by the results of acute, neurotoxicity, immunotoxicity, or developmental and reproductive toxicity tests. For EPA seriously to suggest such benefits for the proposed test rule indicates that it is searching desperately for a rationale for the testing.

EPA also indicates that it would use the data obtained by the test rule to estimate potential risks associated with the accidental release of chemicals as required by Section 112(r) of the Clean Air Act. EDC, VDC, and 1,1,2-trichloroethane, however, are not listed in Section 112(r), nor do they appear in the list of toxic substances and threshold quantities for accidental release prevention that EPA promulgated in accordance with Section 112(r).[35] In fact, only four of the 20 HAPs covered in the proposed test rule have been listed under Section 112(r). EPA cannot justify its proposed test rule on this basis, as the use it identifies is unauthorized by statute.

In addition to the lack of statutory authority for EPA's purported interest in determining the risks of accidental release of EDC, VDC, and 1,1,2-trichloroethane, such a determination is unnecessary as a matter of policy. EPA has already appointed an independent committee to assess the available information on accidental release and develop appropriate policies. In June 1996, EPA's Office of Pollution Prevention and Toxics (OPPT) announced the formation of a 30-member federal advisory committee -- the National Advisory Committee for Acute Guideline Levels for Hazardous Substances -- that would develop and recommend short-term exposure levels for airborne releases of hazardous substances. The Committee, which reports to the Deputy Administrator of EPA, will review toxicological data on over 700 chemicals. The Committee will then prepare acute exposure guideline levels (AEGLs) for high-priority, highly-toxic chemicals. For each chemical, the Committee will adopt three

[35] 40 C.F.R. § 68.130. VDC does appear in the List of Flammable Substances and Threshold Quantities for Accidental Release Prevention published in the same regulation. *Id.* None of the tests proposed for the HAPs relate to flammability, however, and EPA cannot seek to justify its proposal on this basis.

AEGLs for four time periods, ranging from 30 minutes to 8 hours. The three levels are: AEGL-1, where there is chemical detection; AEGL-2, where discomfort is experienced; and AEGL-3, where disability occurs.

AEGLs may be used for emergency planning, or for response and prevention programs related to manufacture, processing, storage, transportation, or the remediation of waste sites. The AEGLs would not carry any compliance obligation unless a federal or state agency were to adopt or incorporate them into regulations or standards. Several agencies have already evidenced an interest in utilizing the standards. The Occupational Safety and Health Administration (OSHA) has indicated that it intends to use them in setting short-term exposure limits for worker safety and for implementing process safety management standards. In addition, the Department of Transportation has stated its intention to use the AEGLs in calculating the initial isolation and protective action distances that it compiles for use by emergency response personnel at chemical spill sites.[36] Moreover, EPA has stated that it may use AEGLs as a basis for adding chemical substances to the list of chemicals that must be reported under Section 302 of the Emergency Planning and Community Right-to-Know Act.[37] This wide array of interests in and potential uses for the AEGLs demonstrate the comprehensive nature of the role that they will serve.

OPPT has explained that the Committee was formed because nine federal agencies and eight state, local, and nongovernment organizations had developed or were considering developing AEGLs. The obvious intent was to preclude unnecessary duplication. In light of the purpose, goals, and capabilities of the Committee, for EPA now to embark on a separate program to estimate the risks associated with accidental release by requiring manufacturers to undertake the considerable private expense of generating the same information that EPA itself has charged the Committee to develop is redundant at best. Such an action would undermine the very goal that the Agency sought to achieve.

Yet another ostensible secondary purpose set forth by EPA for the test rule is to "better inform communities and citizens of toxic chemical hazards in their own localities." This interest is not served by providing a deluge of information about chemical substances with

[36] Chemical Regulation Reporter, June 21, 1996.

[37] *Id.*, 42 U.S.C. § 11002.

minimal human exposure. Such a barrage merely adds to the clutter of information that the public receives every day about aspects of daily life that might conceivably pose a threat to health or safety. The sheer volume of these reports, from both government and nongovernment sources, lessens the likelihood of the public taking heed of any individual warning in particular. By seeking to publicize every chemical with toxic effects as a risk to local health and safety, regardless of the extent of exposure, EPA compounds this trend and threatens to undermine its own credibility. The goal of informing communities would be better served by taking emissions patterns into account and focusing attention on actual, significant threats.

V. EPA HAS ALREADY DETERMINED THAT THE AVAILABLE DATA ARE ADEQUATE TO CONCLUDE THAT EDC DOES NOT PRESENT AN UNREASONABLE RISK TO HEALTH OR THE ENVIRONMENT

EPA has examined EDC in the recent past and found the available data sufficient to support a conclusion about its potential risks to health or the environment. In 1993, OPPT released a Risk Management 2 Lifecycle and Pollution Prevention Assessment ("RM 2") for EDC (Appendix, Tab 20). The goal of the RM 2 was to perform a risk characterization addressing the question of the future public health impact of EDC exposure if facility releases continued over time at the then-current rate. While acknowledging that no model can predict the future, the RM 2 sought "to provide insight into the need for reductions in current EDC releases" and to develop "a meaningful way to examine the hypothetical risks associated with long term EDC releases." [38]

In evaluating the health and environmental hazard for purposes of the risk characterization, OPPT noted that EDC can cause both acute and chronic health effects, that exposure can effect the liver, lungs, kidneys, and nervous system, and that some evidence supports concerns about reproductive and immunologic effects. Nonetheless, OPPT concluded that "the driving health hazard concern for EDC is carcinogenicity."[39] Therefore, rather than

[38] Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, *Risk Management 2 Lifecycle and Pollution Prevention Assessment for 1,2-Dichloroethane (Ethylene Dichloride) CAS# 107-06-2,* (December 6, 1993) ("RM 2") (Appendix, Tab 20), p. 7.

[39] RM 2, p. 15.

sink into paralysis in an effort to obtain all possible information, OPPT focused the RM 2 report on EDC's status as a probable human carcinogen. In looking at cancer effects, an "upper bound estimate" of EDC's potency was used, meaning that the potency is unlikely to be higher and may actually be lower.[40]

With this in mind, OPPT examined the 1990 TRI data on EDC and came to conclusions regarding its potential health risks. Regarding inhalation exposure from stack and fugitive releases, it concluded that the 1990 data gave some cause for concern. Nationwide, it estimated a total population potentially exposed to EDC by inhalation of 84 million people, and projected that 64 cancers would occur in this group over a 70-year period. The vast majority of these cases, however, 57 of the projected 64, would occur in a much smaller population of 5.9 million people. The highest potential for risk existed among subpopulations in closest proximity to facilities emitting EDC, where the exposure concentration is highest.[41]

Due to uncertainties associated with modeling, OPPT did not regard these estimates as definitive statements about the health risks of EDC. It did consider them cause for concern, however, particularly since releases had increased at three of the highest emitting facilities between 1989 and 1990. OPPT concluded that pollution prevention measures to reduce air emissions would be necessary. Therefore, it decided to obtain the 1991 TRI Form R data for the four facilities associated with the highest projected risks. These data showed a reversal in the increasing air releases, as all four facilities reported reductions in releases for 1991.[42] While the 1991 reductions by themselves were relatively modest, OPPT also considered, pursuant to the Pollution Prevention Act of 1990, releases for 1992 and 1993 predicted by the companies. All four facilities predicted further reductions in releases. OPPT concluded that achievement of those reductions would significantly reduce the potential risks posed by continued EDC release. Consequently, the RM 2 team recommendations emphasized promotion of coordination and pollution prevention strategies both within and between

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[40] RM 2, p. 7.

[41] RM 2, p. 30. The risk increased as the projected subpopulation was narrowed. Thus 43 of the total 64 projected cancers would occur in a subpopulation of 429,765 exposed individuals, and 24 projected cancers -- more than a third of the total -- would occur in a subpopulation of just 8,828 persons.

[42] RM 2, p. 8. Further examination revealed that the estimates for one of the facilities were inaccurate and that releases had not decreased.

industry and government in order to achieve the 1993 target reductions identified by the companies. These efforts were apparently successful, as the TRI data for 1993 indicate that the targets were reached or surpassed by three of the four companies, and that the fourth, though falling short of its target, nonetheless achieved a reduction of approximately 14% over 1992.[43]

EPA should apply the same reasoning that it used in preparing the RM 2 to develop the HAP test rule. There, the Agency was willing to draw reasonable conclusions to protect the public health based on the information available to it. It did exactly what it has to date failed to do for the HAP test rule. It considered emissions patterns and exposure levels and used that information to make policy. OPPT based its decision on potential cancer risk, recognizing that the restrictions imposed on a suspected carcinogen would provide ample protection with respect to other endpoints. Using the known data on EDC exposure and potential cancer risk, EPA was able to complete its risk characterization.

The same factors apply to the proposed HAP test rule for EDC. No matter what information is developed regarding any of the endpoints identified in the proposed test rule, EPA's determination will still be driven by carcinogenicity. Since sufficient data have already been developed for that endpoint, there appears to be no utility in testing for the others. The Agency has not explained why it cannot use the same information that it used for the RM 2, supplemented with modeling as necessary, to conduct the as-yet-undefined residual risk determination that it must perform for EDC.

VI. ANY FINAL TEST RULE SHOULD APPLY TO PROCESSORS AS WELL AS MANUFACTURERS

The proposed test rule places the primary responsibility for conducting testing on manufacturers alone. Processors have a secondary responsibility at best, and "would be required to comply with the rule only if directed to do so in a subsequent notice."[44] This is not

[43] The 1992 TRI showed Ferro Corp. with air emissions of EDC of 1,125,410 pounds a year. In 1993, this number had decreased to 964,600 pounds The 1994 TRI showed an even more impressive reduction, down to 583,750 pounds, a reduction of nearly 40 percent.

[44] 61 Fed. Reg. at 33178. In amending the proposed test rule, EPA specified that only manufacturers which produce more than 25,000 pounds of an HAP in the fiscal year preceding the final test rule will be required to "initially comply" with the test requirements, and that processors and manufacturers producing less than 25,000

an equitable distribution of the burden. Processing a chemical substance often gives rise to levels of emissions orders of magnitude greater than the manufacturing process, which is largely enclosed. Responsibility for complying with the test rule should be apportioned among manufacturers and processors in accordance with their respective emissions of the HAP in question.

In the case of EDC, the emissions analysis set forth by OPPT in the RM 2 discussed above shows that the proposed HAP test rule misapportions the responsibility for testing. The RM 2 notes that the manufacture of EDC and its conversion into associated products is the largest use of the substance, producing 17 billion pounds and processing 99% of the 16 billion pounds consumed domestically each year.[45] Most EDC -- about 93% of domestic product -- is used in the production of vinyl chloride monomer (VCM). VCM is itself used to make polyvinyl chloride resins. This process is tightly controlled, and EDC is largely consumed in production, with little left over for emissions. This enables the manufacturers and processors of EDC to hold releases to low levels. Consequently, as OPPT noted, "[t]he EDC manufacturing sector is relatively efficient at minimizing environmental release of EDC." Indeed, only one of the top seven release facilities was a manufacturer.[46]

The other six top release facilities in the RM 2 came from what OPPT termed the "miscellaneous sector" of the industry, *i.e.*, the use of EDC in producing such products as adhesives and coatings, polysulfide elastomers, grain fumigants, lacquer solvents, and paint and varnish. This sector of the industry is, in OPPT's phrase, "relatively inefficient." A review of the production numbers and exposure data reveals the magnitude of that inefficiency. While miscellaneous uses involve less than 1% of the total production volume of EDC, they are responsible for 73% of the 1989 TRI releases.[47]

The proposed HAP test rule, however, takes no notice of the disproportionately large share of emissions attributed to processors. Instead of seeking to apportion responsibility to

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pounds will only be required to comply if there are no manufacturers that initially comply. 62 Fed. Reg. 67466 (December 27, 1997).

[45] RM 2, p. 3.

[46] RM 2, p. 5. That manufacturer, PPG Industries, Inc., has since dramatically reduced its emissions of EDC. Its 1994 TRI levels were approximately 20% of the 1990 TRI levels cited in the RM 2.

[47] RM 2, p. 65.

those most responsible for emissions, the proposal would place the primary (if not entire) obligation for conducting and paying for the testing on manufacturers. As noted above, processors, including those involved in the miscellaneous sector of the industry, would only be responsible for complying with the test rule if EPA requires them to in a subsequent notice. This is an affront to even the most basic concept of fairness. Processors create the vast majority of emissions, while manufacturers, which are responsible for only a minor portion of total emissions, are forced by EPA to assume the burden of determining the health effects of those emissions. The testing costs should be shared by those firms responsible for release to the environment.

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TABLE A

1,1,2-Trichloroethane Worker Exposure Survey

Company	Number of Workers Potentially Exposed
Manufacturers:	
CONDEA Vista Co.	42
Dow Chemical Co.	80
Formosa Plastics Corp.	16
Georgia Gulf Corp.	44
Occidental Chemical Corp.	36
PPG Industries, Inc.	125
Vulcan Materials Co.	44
Westlake Monomers Corp.	13
TOTAL:	**400**
Users:[1]	
User #1	12
User #2	21
User #3	20
User #4	1
User #5	18
User #6	4
TOTAL:	**76**
GRAND TOTAL:	**476**

[1] Only one U.S. company manufactures 1,1,2-trichloroethane for sale to customers. The identity of those customers is proprietary information.

TABLE B

Vinylidene Chloride (VDC) Worker Exposure Survey

Company	Number of Workers Potentially Exposured
Applied Extrusion Tech's Inc.	6
ARCO Chemical Company	20
B.F. Goodrich Company	40
The Dow Chemical Company	136
DuPont	33
Eastman Kodak Company	40
Hampshire Chemical Corp.	18
LaRoche Industries, Inc.	12
3M	50
Morton Int'l Inc.	130
PPG Industries, Inc.	125
Solutia	100
TOTAL:	**710**

Table 1
Comparison of Candidate Studies to OPPTS Test Guidelines for Subchronic Toxicity

		Candidate Studies for Route Extrapolation	
	Study:	White et al. 1985	NCI 1978
	Subchronic effects observed:	Liver, hematological	Lack of histopathological changes
	LOAEL:	44-46 mg/kg-day	None
	NOAEL:	3.9-4.4 mg/kg-day	400 mg/kg-day [CHECK]
Parameter	**Recommended**		
Test species	Rat (other mammal)	Mouse	Mouse
Strain	Common lab	CD-1	B6C3F1
Age	Young, healthy	Young, healthy	Young, healthy
Sex	Both	Both	Both
Number of animals	10/sex/dose, more if interim sacrifice	32-48/sex/group	50/sex/dose
Husbandry	Standard	Adequate	Some limitations, but adequte overall
Control Groups	Concurrent sham or vehicle control	Vehicle control	Vehicle and untreated controls
Concentration level and selection	> =4 (including control)	Male (0, 4.4, 46, and 305 mg/kg-day); Female (0, 3.9, 44, and 384 mg/kg-day)	0, 195, 390 mg/kg-day
Limit dose	Maximum tolerable dose achieved	Range determined based on 1-day, and 14-day range finding studies	Range determined based on 6 week study
Intermediate dose	Provides gradiation of effects	Adequate	NA
Lowest dose level	Should provide NOAEL	Adequate	Adequate
Administration of the substance	6 hr/day, 7 day/week	Daily, 90 days	5 d/wk, 78 wks
Observation period	90 days	90 days	630 days
Exposure specifications and physical measurements	Nose only or whole body	Oral (drinking water)	Oral (oil gavage)
Observation of animals	Mortality (daily), clinical (weekly)	Adequate	Adequate
Clinical pathology	Hematology and clinical chemistry	Adequate	Not evaluated
Ophthalmological examinations	High-dose and control	Not applicable	Histopathological examination of ocular tissues
Gross pathology	Gross necropsy and organ weights	Adequate	Adequate
Histopathology	Histopathology, including respiratory tract	Not evaluated	Dated methods, but adequate overall
Results reporting	Tabular results, statistics	Tabular means & SDs, Duncan's multiple range test	Tabular incidences
Evaluation	Adequately described	Adequate	Adequate

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Table 2
Comparison of Candidate Studies to OPPTS Test Guidelines for Neurotoxicity

		Candidate Studies for Route Extrapolation		
		Study: White et al. (1985) - Acute	NCI (1978) - Longer term	NCI (1978) - Longer term
		Neurological effects observed: Sedation, loss of righting reflex	Lack of histopathological changes for brain and nerves	Lack of histopathological changes for brain and nerves
		LOAEL: 450 mg/kg-day	None	None
		NOAEL: 400 mg/kg-day	390	92
Parameter	**Recommended**			
Test species	Rat (mice or dog)	Mouse	Mouse	Rat
Age	Young adult (>42 d)	Young adult	Young adult	Young adult
Sex	Both	Both	Both	Both
Number of animals	10/sex/dose	8/sex/dose	50/sex/dose	50/sex/dose
Control Groups	Concurrent sham or vehicle control	None	Vehicle and untreated controls	Vehicle and untreated controls
Concentration level and selection	>=4 (including control)	7 dose groups between 200 and 600 mg/kg	0, 195, 390 mg/kg-day	0, 46, 92 mg/kg-day
Acute studies	MTD; < 2g/kg	Adequate	NA	NA
Subchronic and chronic studies	MTD; <1 g/kg	NA	Range determined by 6 week study	Range determined by 6 week study
Administration of the substance	Appropriate route of exposure	Oral (water gavage)	Oral (oil gavage)	Oral (oil gavage)
Combined protocol	Combine with other endpoints	NA	NA	NA
Time of testing	Acute (0, 8hr, 7d, 14 d); subchronic (0, 4 wk, 8 wk, 13 wk)	up to 14 days	Functional tests not evaluated	Functional tests not evaluated
Functional observational battery	Standard evaluations for appearance, behavior, and functional integrity	Appearance and behavior	Appearance and behavior evaluated grossly	Appearance and behavior evaluated grossly
List of measures	Autonomic function, abnormal motor movements, response to general and sensory stimuli, alertness, grip strength, landing foot splay, body weight, behavioral changes	Abnormal motor movements	Not evaluated	Not evaluated
Motor activity	Individually assessed, automated	Not evaluated	Not evaluated	Not evaluated
Neuropathology	Neuropathological examinations	Gross necropsy only	Adequate	Adequate
Results	Tabular, per animal	Text only	Tabular incidences	Tabular incidences
Evaluation	Adequately described, statistics	Limited	Adequate	Adequate

Table 3

Comparison of Candidate Studies to OPPTS Test Guidelines for Carcinogenicity

		Candidate Studies for Route Extrapolation	
		Study: NCI (1978) - Mouse study	NCI (1978) - Rat study
		Carcinogenic effects observed: Liver, adrenal tumors	None
Parameter	**Recommended**		
Test species	Rats and mice	Mouse	Rat
Strain	Common lab strain	B6C3F1	Osborne-Mendel
Age	Young, healthy (<8 wks)	Young	Young
Sex	Both	Both	
Number of animals	50/sex/group	50/sex/group	50/sex/group
Control Groups	Concurrent sham or vehicle control	Vehicle and untreated controls	Vehicle and untreated controls
Concentration level and selection	>=4 (including control)	0, 195, 390 mg/kg-day	0, 46, 92 mg/kg-day
Limit dose	MTD; <1000 mg/kg-day	Adequate	May not have been achieved
Intermediate dose	Provides gradation of effects	Adequate	Adequate
Lowest dose level	Provides NOAEL	NA	NA
Route	Inhalation	Oral (oil gavage)	Oral (oil gavage)
Exposure	6 hr (inhalation), 5-7 d/wk for 18 (mice) or 24 (rat) months	5 d/wk, 78 wk	5 d/wk, 78 wk
Observation period	Lifespan	630 days	777 days
Observation of animals	Morbidity (daily), clinical (weekly)	Adequate	Adequate
Clinical pathology	at 12 and 18 months	Not evaluated	Not evaluated
Immunotoxicity screen	Optional	NA	NA
Gross necropsy	Complete	Adequate	Adequate
Histopathology	Complete	Adequate	Adequate
Results reporting	Tabular, per animal, statistics	Incidence	Incidence
Evaluation reporting	Adequately reported	Adequate	Adequate

Table 4

Comparison of Candidate Studies to OPPTS Test Guidelines for Immunotoxicity

		Candidate Studies for Route Extrapolation	
	Study:	Sanders et al. (1985); White et al. 1985	NCI (1978)
	Immunological effects observed:	Humoral immune system depression	Lack of histopathology
	LOAEL:	44-46 mg/kg-day	None
	NOAEL:	3.9-4.4 mg/kg-day	390 mg/kg-day
Parameter	**Recommended**		
Test species	Rat or mouse	Mouse	Mouse
Strain	Common lab strain	CD-1	B6C3F1
Age	Young, healthy (6-8 wks)	Young, healthy	Young, healthy
Sex	Both	Both	Both
Number of animals	6 - 10/sex/group	32-48/sex/dose	50/sex/dose
Husbandry	Standard	Adequate	Adequate
Control & Test Substances	Purity and vehicle evaluated	95%	92.70%
Control Groups	Concurrent sham or vehicle control	Vehicle control	Vehicle and untreated controls
Dose administration	Inhalation	Oral (drinking water)	Oral (oil gavage)
Concentration level and selection	> =4 (including control)	Male (0, 4.4, 46, and 305 mg/kg-day); Female (0, 3.9, 44, and 384 mg/kg-	0, 195, 390 mg/kg-day
Limit dose	MTD	Range determined based on 1-day, and 14-day range finding studies	Range determined based on 6 week study
Lowest dose level	Provides NOAEL	Adequate	Adequate
Administration of the substance	30-90 days; 5-7 days/week	90 days	5 d/wk, 78 wks
Observation period	30-90 days	90 days	630 days
Immunotoxicity tests	Functional (antibody plaque forming cell assay or immunoglobin quantification); Enumeration of splenic or peripheral blood T cells, B cells and NK cells	Complete	Histopathology of lymph nodes, spleen, bone marrow
Results reporting	Tabular, statistics	Tabular means & SD, Duncan's multiple range test	Tabular incidences
Evaluation repoting	Adequately described	Adequate	Adequate

Table 5
Comparison of Candidate Studies to OPPTS Test Guidelines for Acute Toxicity

Candidate Study for Route Extrapolation

Study: Daniel et al. (1994)
Subchronic effects observed: Lack of histopathological effects
LOAEL: None
NOAEL: 100

Parameter	Recommended	
Test species	Rat or mouse	Rat
Strain	F344 or B6C3F1	Sprague-Dawley
Age	Young adult	Young adult
Sex	Both	Both
Health Status	Evaluate initially	Adequate
Number of animals	5 /sex/dose	10/sex/dose
Control Groups	Concurrent sham or vehicle control	Vehicle control
Concentration level and selection	>=4 (including control)	0, 10, 30, 100, 300 mg/kg-day
Limit dose	MTD or 5 mg/L	Adequate
1-hr study	If triggered, >=3	Not applicable
8-hr study	If triggered, >=3	Not applicable
Exposure	Nose only or whole body	Oral (oil gavage)
Environmental conditions	~22°C; 40 - 60% humidity	Adequate
Exposure periodicity	4 hr (1 & 8hr, if triggered)	1x/d, 10 d
Physical measurements	Environmental conditions monitored	Adequate
Observation period	24 hr	10 days
Gross pathology	Full necropsy, organ weights	Adequate
Histopathology	Histopathology, including respiratory tract	Adequate
Bronchoalveolar lavage	Provide indicators of lung damage	Not evaluated
Equipment and test methods reporting	Adequately defined	Adequate
Results reporting	Tabular results, per animal, statistics	Adequate

Table 6

Comparison of Candidate Studies to OPPTS Test Guidelines for Subchronic Toxicity

		Candidate Studies for Route Extrapolation			
		Study: NTP (1991) - Drinking water study in rats	NTP (1991) - Corn oil gavage study in rats	NTP (1991) - Drinking water study in mice	Daniel et al. (1994)
		Subchronic effects observed: Mild renal tubular regeneration, increased kidney	Necrosis of the thymus	Mild-to-moderate renal tubular regeneration	Liver and kidney weight changes, hematological effects
		LOAEL: 102	240	2710	75
		NOAEL: 58	120	781	37.5
arameter	**Recommended**				
est species	Rat (other mammal)	Rat	Rat	Mouse	Rat
rain	Common lab	F344/N, Sprague-Dawley, Osborne Mendel	F344/N	B6C3F1	Sprague-Dawley
ge	Young, healthy	Young, healthy	Young, healthy	Young, healthy	Young, healthy
ex	Both	Both	Both	Both	Both
umber of animals	10/sex/dose, more if interim sacrifice	10-20/sex/dose	10/sex/dose	10/sex/dose	10/sex/dose
usbandry	Standard	Adequate	Adequate	Adequate	Adequate
ontrol Groups	Concurrent sham or vehicle control	Untreated	Vehicle	Untreated	Vehicle
oncentration level and selection	> =4 (including control)	0, 49-82, 86-126, 147-213, 259 428, 515-727 mg/kg-day	0, 30, 60, 120, 240, 480 mg/kg day (males); 0, 18, 37, 75, 150, 300 mg/kg-day (females)	0, 249, 448, 781, 2710, 4207 mg/kg-day (males); 0, 244, 647, 1182, 2478, 4926 mg/kg-day (females)	0, 37.5, 75, 150 mg/kg-day
imit dose	Maximum tolerable dose achieved	Adequate	Adequate	Adequate	Adequate
ntermediate dose	Provides gradiation of effects	Adequate	Adequate	Adequate	Adequate
owest dose level	Should provide NOAEL	Adequate	Adequate	Adequate	Adequate
dministration of the substance	6 hr/day, 7 day/week	Daily	Daily	Daily	Daily
bservation period	90 days	13 weeks	13 weeks	13 weeks	90 days
xposure specifications and physical easurements	Nose only or whole body	Oral (drinking water)	Oral (oil gavage)	Oral (drinking water)	Oral (oil gavage)
bservation of animals	Mortality (daily), clinical (weekly)	Adequate	Adequate	Adequate	Adequate
linical pathology	Hematology and clinical chemistry	Adequate	Adequate	Adequate	Adequate
phthalmological examinations	High-dose and control	Necropsy and histology of eyes	Necropsy and histology of eyes	Necropsy and histology of eyes	
ross pathology	Gross necropsy and organ weights	Adequate	Adequate	Adequate	Adequate
istopathology	Histopathology, including respiratory tract	Adequate	Adequate	Adequate	Adequate
esults reporting	Tabular results, statistics	Tabular means. standard deviations, incidences	Tabular means. standard deviations, incidences	Tabular means. standard deviations, incidences	
valuation	Adequately described	Adequate	Adequate	Adequate	

Table 7
Comparison of Candidate Studies to OPPTS Test Guidelines for Neurotoxicity

		Candidate Studies for Route Extrapolation	
	Study	NTP 1991 (drinking water study)	NTP 1991 (oil gavage study)
	Neurological Effects Observed	None	Tremors, necrosis of the cerebellum
	LOAEL	NA	240 mg/kg-day
	NOAEL	492 mg/kg-day	120 mg/kg-day
Parameter	**Recommended**		
Test species	Rat (mice or dog)	Rat	Rat
Strain	Common	F344/N, Sprague-Dawley, Osborne Mendel	F344/N
Age	Young adult (>42 d)	42 d	42 d
Sex	Both	Both	Both
Number of animals	10/sex/dose	10-20/sex/dose	10-20/sex/dose
Control Groups	Concurrent sham or vehicle control	Vehicle control	Vehicle control
Concentration level and selection	>=4 (including control)	0, 49-82, 86-126, 147-213, 259-428, 515-727 mg/kg-day	Male (0, 30, 60, 120, 240, or 480 mg/kg-day); Female (0, 18, 37, 75, 150, or 300 mg/kg-day)
Dose selection (acute)	MTD; < 2g/kg	NA	NA
Dose selection (subchronic)	MTD; <1 g/kg	Adequate	Adequate
Administration of the substan	Appropriate route of exposure	Oral	Oral
Combined protocol	Combine with other endpoints	NA	NA
Time of testing	Acute (0, 8hr, 7d, 14 d); subchronic (0, 4 wk, 8 wk, 13 wk)	90 days	90 days
Functional observational batt	Standard evaluations for appearance, behavior, and functional integrity	Clinical signs evaluated weekly	Clinical signs evaluated weekly
List of measures	Autonomic function, abnormal motor movements, response to general and sensory stimuli, alertness, grip strength, landing foot splay, body weight, behavioral changes	Abnormal body movements (tremor), body weight	Abnormal body movements (tremor), body weight
Motor activity	Individually assessed, automated	Not evaluated	Not evaluated
Neuropathology	Neuropathological examinations	Brain, sciatic (if signs present)	Brain, sciatic (if signs present)
Results	Tabular, per animal, statistics	Incidence, mean & SD, Dunn's or Shirley's tests	Incidence, mean & SD, Dunn's or Shirley's tests
Evaluation	Adequately described	Adequate	Adequate

APPENDIX

CONTAINS NO CBI

File: ATSDR -
1,1,2-TRICHLOROETHANE

REV 8(E)

1,1,2-TRICHLOROETHANE

Agency for Toxic Substances and Disease Registry
U.S. Public Health Service

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5. POTENTIAL FOR HUMAN EXPOSURE

5.1 OVERVIEW

1,1,2-Trichloroethane is predominantly a man-made chemical whose presence in the environment results from anthropogenic activity. This chemical has also been identified as an intermediate in the biodegradation of 1,1,2,2-tetrachloroethane, another man-made chemical. It is made commercially by the chlorination of ethylene with chlorine or by the oxychlorination of ethylene with HCl and oxygen. It is primarily used as a captive intermediate in the manufacture of 1,1-dichloroethene (vinylidene chloride), but may also be used as a solvent, especially in chlorinated rubber manufacture. Production and use information are proprietary, however effluent monitoring data indicate that high levels (>100 ppb) of discharge are associated with laundries, and the organic chemicals and mechanical products industries (Table 5-1). The maximum levels in these wastewaters were 109 - 250 ppb. Gaseous releases include vent gas and fugitive emissions from the production and use of 1,1,2-trichloroethane as well as volatilization from wastewater and municipal treatment plants. Releases to soil are expected to involve the landfilling of sludge and process residues. Thus far, 1,1,2-trichloroethane has been found at 45 of 1177 hazardous waste sites on the National Priorities List (NPL) in the United States (VIEW Database 1989). Based on the release pattern of other chlorinated ethanes and ethenes, it is expected that the release pattern for 1,1,2-trichloroethane is 70-90% to air, 10-30% to land, and a few percent to water. No use with significant consumer and general population exposures has been identified.

If 1,1,2-trichloroethane is released into soil, it is expected to partially leach into the subsurface and groundwater (because it has a low soil adsorption coefficient), and to partially volatilize. In groundwater, it will be subject to anaerobic biodegradation, however no information concerning reaction rates is available. Biodegradation is expected to occur in sediment and landfills when anaerobic conditions are present. The mechanism for biodegradation is reductive dehalogenation, which leads to the formation of vinyl chloride, a human carcinogen (USDHHS 1985). From the limited data available, biodegradation under aerobic conditions, such as exists in surface soil, will be very slow, at best. In surface water, volatilization is the primary fate process (half-life 4.5 hr in a model river). Adsorption to sediment, bioconcentration in aquatic organisms, aerobic biodegradation, and hydrolysis are thought to be negligible by comparison. In the atmosphere, the dominant removal process is expected to be oxidation by photochemically-generated hydroxyl radicals, which proceeds by H-atom abstraction (estimated half-life 49 days). The radical so produced subsequently reacts with atmospheric oxygen and other atmospheric species. Removal from the atmosphere is also thought to occur from washout by precipitation; however, most of the 1,1,2-trichloroethane removed by this process is expected to reenter the atmosphere by volatilization. Because oxidation in the atmosphere is slow, considerable dispersion of

5. POTENTIAL FOR HUMAN EXPOSURE

TABLE 5-1. Sources of 1,1,2-Trichloroethane Effluents[a]

Industry	Frequency	Concentration (ppb)		
		Maximum	Medium	Low
Timber products	1	18.46	18.46	18.46
Organics and plastics	1	7.12	7.12	7.12
Inorganic chemicals	2	6.00	4.00	2.01
Plastics and synthetics	2	31.85	3.65	0.26
Auto and other laundries	1	108.99	108.99	108.99
Organic chemicals	1	203.77	203.77	203.77
Mechanical products	4	249.52	45.74	1.33
Transportation equipment	3	75.33	66.34	24.53
Synfuels	1	2.43	2.43	2.43
Publicly owned treatment works	4	15.22	1.20	0.42

[a]Discharges to water
Source: Shackelford et al. 1983

1,1,2-trichloroethane from source areas would be expected to occur. Thus, it is conceivable that 1,1,2-trichloroethane could be transported from other countries where it may be more widely used.

The general population may be exposed to low levels of 1,1,2-trichloroethane through inhalation of contaminated ambient air. Limited monitoring data suggest that roughly one-quarter to one-half of the urban population may be so exposed. Where 1,1,2-trichloroethane is found, levels appear to be about 10-50 ppt. Results from a nationwide monitoring study of grcundwater supplies show that exposure to 1,1,2-trichloroethane from contaminated drinking water appears to be uncommon (Westrick et al. 1984). However, in a New Jersey survey, 6.7% of the wells contained detectable levels of 1,1,2-trichloroethane; the most polluted wells being associated with urban land use (Page 1981; Greenberg et al. 1982). It is difficult to assess occupational exposure because data on current production and use are unavailable. A National Occupational Exposure Survey (NOES) by the National Institute of Occupational Safety and Health (NIOSH) through May 1988, estimates that 1,036 employees are potentially exposed to 1,1,2-trichloroethane in the United States. Occupational exposure will be primarily via inhalation.

5.2 RELEASES TO THE ENVIRONMENT

5.2.1 Air

1,1,2-Trichloroethane is emitted in vent gas when produced by the oxychlorination of ethylene dichloride (Liepins et al. 1977). Environmental releases of 1,1,2-trichloroethane from 1,1-dichloroethene manufacture are small; an EPA study found no 1,1,2-trichloroethane in process vent gas (Thomas et al. 1982). 1,1,2-Trichloroethane is formed in small quantities and may be released in vent gas or fugitive emissions during the production of other chlorinated hydrocarbons, for example, 1,2-dichloroethane and 1,1,1-trichloroethane (Thomas et al. 1982). Fugitive emission from its use as a solvent and volatilization from wastewater constitute the major environmental release of 1,1,2-trichloroethane. An estimate of the total release of 1,1,2-trichloroethane was made for 1979 by comparing ambient levels of 1,1,1-trichloroethane and 1,1,2-trichloroethane in urban air and releases of 1,1,1-trichloroethane (Thomas et al. 1982). The annual amount of 1,1,2-trichloroethane released annually was calculated to be 10,000-20,000 million tons.

A correlation of data from the EPA Air Toxics Emission Inventory with industrial source categories (SIC codes), shows that volatile emissions of 1,1,2-trichloroethane are associated with plastic materials and resins, industrial organic chemicals, petroleum refining, gaskets-packing and sealing devices, plating and polishing, residential lighting fixtures, radio and TV communication equipment, electronic components, motor vehicles parts and accessories, engineering and scientific instruments, photographic

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IMMUNOTOXICOLOGICAL INVESTIGATIONS IN THE MOUSE: GENERAL APPROACH AND METHODS

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ABSTRACT

The adverse effects of chemicals on the lymphoreticular system have generated considerable toxicological interest. In this series of papers, the effects of selected environmentally relevant compounds are reported. This first paper describes the methods and general approach used in judging a chemical's potential risk to the immune system. Risk evaluation was approached utilizing acute, 14- and 90-day studies. Both sexes of the CD-1 random-bred mouse were employed. The immune system was evaluated against a background of more standard toxicological parameters, which included fluid consumption, body and organ weights, hematology, serum and liver chemistries, hepatic microsomal enzyme activities and blood coagulation. Bone marrow status was evaluated by assessing DNA synthesis. Humoral immunity was evaluated by determining the number of IgM spleen antibody-forming cells (AFC) to sheep erythrocytes (sRBC), the serum antibody level to sRBC, and spleen lymphocyte response to the B cell mitogen, lipopolysaccharide (LPS). The status of cell-mediated immunity was assessed by quantitating the delayed type hypersensitivity (DTH) response to sRBC, proliferation of the popliteal lymph node, and the spleen cell response to the T lymphocyte mitogen, Concanavalin A (Con A). Macrophage function was evaluated by measurement of the vascular clearance rate and distribution of radiolabeled sRBC in the liver, spleen, lungs, and thymus, and

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recruitability, adherence, chemotaxis, and phagocytic activity of peritoneal exudate cells (PEC). Historical control data from six 14- and 90-day studies conducted over a one year period are given. The data resulting from these types of studies can provide a basis for the initial evaluation of a chemical's adverse effect on the immune system.

INTRODUCTION

The adverse effects of chemicals on the organs, tissues, and cells of the lymphoreticular system have received considerable attention from both toxicologists and immunologists. This interest is well founded since the immune system has been shown to be the target organ of certain chemically and physically diverse compounds[1,2,3]. This system, like all other systems in the body, is complex, with several types of cells working both independently and in concert, to carry out a role in homeostasis. Immunotoxicology is a subject gaining increased activity and awareness because the physiology and biochemistry of the immune system are now being systematically dissected; as a result, the basic processes involved in host defense mechanisms are better understood. A second reason for the interest in immunotoxicology is that it represents a system in which the cells from exposed animals can be readily removed and their function(s) examined in vitro. This is in keeping with the direction that toxicology is taking, i.e., complementing morphological changes with functional alterations.

This paper describes the approach we have taken in evaluating the immunotoxicity of chemicals in experimental random-bred mice. The effects of a given chemical on the immune system were investigated against a background of standard toxicological procedures. In this way, the specificity of the immune system as a target could be more clearly defined. Historical control data are provided for each of the assays, with the data presented as the mean ± SD. In addition, an analysis of variance was calculated on the combined experiments to determine if there were significant differences among the control values from experiment to experiment.

EXPERIMENTAL APPROACH

Figure 1 outlines the experimental design which is now being used to evaluate chemicals for their target organ toxicity.

After the compound was selected for study, unknown physical and chemical properties of the chemical were determined and necessary studies were performed to assure appropriate integration into the in vivo systems. These data included: confirmation of identity, solubility properties, pH characteristics, stability at concentrations in the test system, and purity.

Acute toxicity studies were performed on eight week old male and female mice using the route of administration which most closely approximates human exposure. Routinely, eight mice were used per group and at least six dose levels were employed. In many of these studies, we were concerned with environmental chemicals that are introduced orally. The mice were exposed via an 18 gauge stainless steel stomach tube after 18 hr of fasting. The mice were observed hourly for the first eight hours for behavioral changes and morbidity, then twice daily thereafter for 14 days. The animals still alive at the end of the experimental period were sacrificed, and all mice were necropsied and examined for gross pathology. Calculations of the LD_{50} and slopes of the dose-response curves with 95% confidence limits were performed by the Probit Procedure of Goodnight[4]. These data provided the basis for selecting the dose levels employed in the 14- and 90-day studies, and began to indicate the possible target organ(s).

Following the acute studies, a 14-day range finding study was performed. If no sex differences were seen in the acute toxicity, only male mice were used. Again, the relevant route

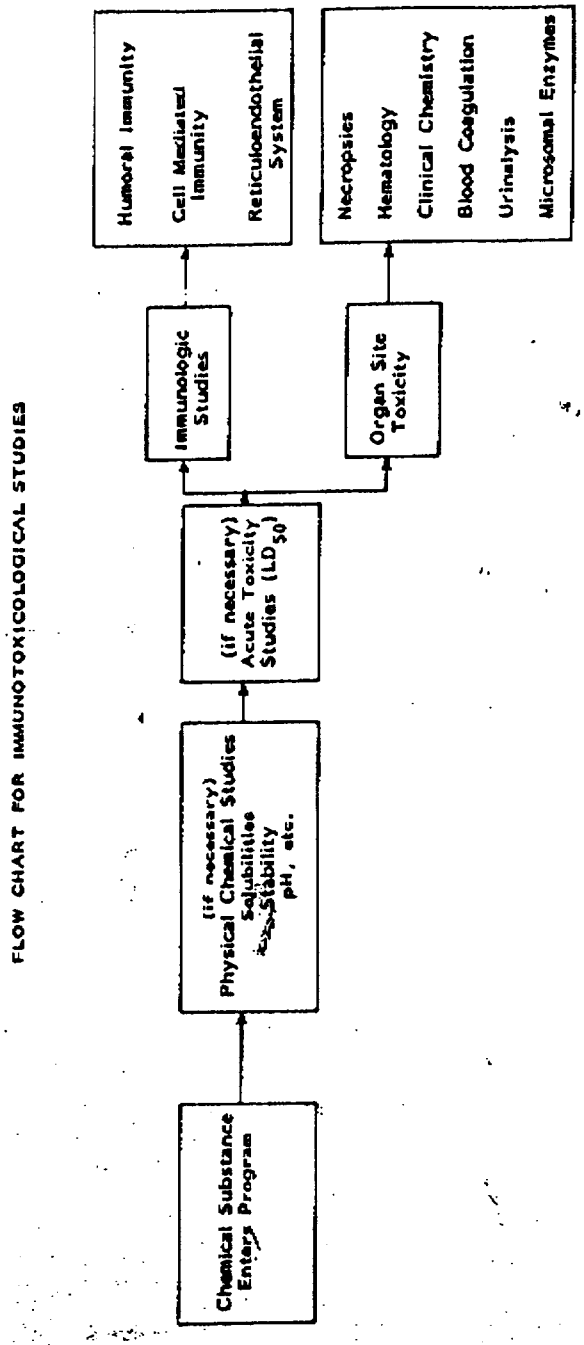

FIG. 1. Flow chart for immunotoxicological studies.

of exposure was employed. The chemical was administered daily for 14 days via stomach tube at two dose levels. These levels were usually 1/10 and 1/100 the LD_{50}. Body weights were determined and recorded prior to the initiation of exposure, and again one and two weeks after exposure.

Table 1 lists the variables monitored after 14 days of exposure. Three sets of mice were used to obtain this toxicological information. In one set of mice, humoral immunity was assessed by enumerating the number of spleen IgM AFC to sRBC, both as a function of AFC per spleen and per 10^6 spleen cells. In the same mice, serum obtained from blood collected by cardiac puncture was used for serum chemistry studies. These included glutanic-pyruvic transaminase, lactate dehydrogenase and blood urea nitrogen. A second set of mice was used for general toxicological assessment. Cardiac blood was collected into 3.2% sodium citrate (1:10 citrate to blood) for hematology and coagulation studies. Hematological studies included leukocyte counts, hematocrit, and hemoglobin levels. If a change was seen in leukocyte counts, a differential analysis was performed. A complete necropsy was performed on this set of mice and the following organs were removed, trimmed, and weighed: brain, liver, spleen, lungs, thymus, kidneys, and testes. The organ weight data were expressed as both organ to body weight ratio and as organ to brain weight ratio. Cell-mediated immunity was assessed in the third set of mice by measurement of the DTH response to sRBC.

Tables 2-6 show the control values for CD-1 mice in the 14-day studies. These values were derived from six 14-day studies performed over a one-year period. The mice were five weeks of age when they arrived at our facility. They were quarantined for seven days and then placed in the study for the two-week period. The 14-day study provided baseline data for the design of the 90-day study. Except for mutagenic, carcinogenic, teratogenic, and reproductive effects, the 90-day study should successfully describe the target organ toxicity.

TABLE 1
Parameters Measured in a 14-Day Study[a]

Standard Toxicology

1. Body Weights
2. Necropsy - Gross Pathology
3. Organ Weights
4. Hematology
5. Serum Chemistries
6. Blood Coagulation

Immunotoxicology

1. Humoral immunity - spleen AFC response to sRBC
2. Cell-mediated immunity - DTH response to sRBC

[a]Doses were based on the acute toxicity study and were usually 1/10 and 1/100 of LD50.

TABLE 2
Control Values for Body Weights (Grams) of Two Month Old Male CD-1 Mice[a]

Final Weight	Initial Weight	Change in Weight
30.0 ± 2.8	24.2 ± 2.3	5.8 ± 2.3

[a]Values represent the mean ± SD of 334 control male CD-1 mice derived from six 14-day experiments. All weight means were significantly different ($p < 0.05$) among the six experiments.

Table 7 lists the variables that were measured during, and at the conclusion of, the 90-day study. Six sets of mice were used to evaluate these variables. The first set was used for necropsy (gross pathology), organ weights, microsomal mixed functional oxidase parameters, liver glutathione levels, hematology, coagulation, hemagglutination titer to sRBC and bone marrow DNA synthesis. The second set of mice was used to

TABLE 3
Control Organ Weight Values of Two Month Old Male CD-1 Male[a]

Organ	Weight (mg)	% Body Weight	Organ/Brain
Brain	440 ± 39	1.47 ± 0.15*	-
Liver	1862 ± 294*	6.17 ± 0.73	4.26 ± 0.74
Spleen	170 ± 53	0.56 ± 0.17	0.39 ± 0.12
Lungs	214 ± 35	0.71 ± 0.11	0.48 ± 0.07
Thymus	79 ± 19	0.26 ± 0.06	0.18 ± 0.04*
Kidney	522 ± 84	1.73 ± 0.20	1.19 ± 0.15
Testes	211 ± 34	0.70 ± 0.11	0.48 ± 0.07*

[a]Values represent the mean ± SD of 68-70 control male CD-1 mice derived from six 14-day experiments. All values were significantly different ($p < 0.05$) among the six experiments, except where indicated by an asterisk.

TABLE 4
Control Values for Selected Hematological, Serum Chemistry and Blood Coagulation Parameters in Two Month Old Male CD-1 Mice[a]

Parameter	Values
Hemoglobin (g%)	12.7 ± 2.1
Hematocrit (%)	41.2 ± 2.5
Leukocytes ($10^3/mm^3$)	7.44 ± 2.76
Lactate Dehydrogenase (IU/L)	926 ± 195
Glutamic-Pyruvic Transaminase (IU/L)	56.4 ± 21.6
Blood Urea Nitrogen (mg%)	27.2 ± 4.8
Prothrombin Time (sec)	8.4 ± 0.8
Fibrinogen (mg%)	298 ± 41

[a]Values represent the mean ± SD derived from 68 control mice used in six 14-day studies. All values were significantly different ($p < 0.05$) among the six experiments.

TABLE 5
Control Values for Humoral Immune Assessment in Two Month Old Male CD-1 Mice[a]

Parameter	Values
Spleen Weight (mg)	204 ± 47
Spleen Cell Number ($\times 10^{-8}$)	1.65 ± 0.48
IgM AFC/Spleen ($\times 10^{-5}$)	4.86 ± 3.18
IgM AFC/10^6 Spleen Cells	2904 ± 1667

[a]Values represent the mean ± SD derived from 68 control mice in six 14-day studies. The antibody forming cells were enumerated on the peak day of response (day 4). All parameters were significantly different ($p < 0.05$) among the six experiments.

TABLE 6
Control Values for the DTH Response to sRBC in Two Month Old Male CD-1 Mice[a]

Parameter	Value
Stimulation Index	3.73 ± 1.60

The value represents the mean ± SD derived from 70 control mice used in six 14-day studies. A value of 1.97 has been subtracted from each animal value to correct for non-specific swelling as described in Methods. There was no significant difference ($p < 0.05$) among the six experiments.

determine spleen AFC response to sRBC, spleen cell response to mitogens, and serum chemistries. Assessment of cell-mediated immunity, as measured by the DTH response to sRBC, was accomplished with the third set of mice, while the fourth set of mice was used to determine popliteal lymph node proliferation. The fifth set of mice was used to measure the functional activity of

TABLE 7
Parameters Measured in a 90-Day Study

A. Standard Toxicology

1. Body weights – twice weekly
2. Fluid consumption
3. Necropsy – gross pathology
4. Organ weights – brain, liver, spleen, lungs, thymus, kidneys, and testes
5. Hematology – hematocrit, erythrocytes, leukocytes, differential, platelets, and hemoglobin
6. Coagulation – prothrombin time, activated partial thromboplastin time, and fibrinogen
7. Serum chemistries – lactate dehydrogenase, glutamic-pyruvic transaminase, glutamic-oxaloacetic transaminase, alkaline phosphatase, blood urea nitrogen, total protein, glucose, cholesterol, creatinine, phosphorus, calcium, sodium, chloride, and potassium
8. Liver chemistries – glutathione levels

B. Hepatic Microsomal Mixed Functional Oxidase Parameters

1. Liver weight
2. Microsomal protein
3. P450 content
4. Cytochrome b_5
5. Aminopyrine-N-demethylase
6. Aniline hydroxylase

C. Cell Mediated Immunity

1. DTH response to sRBC
2. Popliteal lymph node proliferation to sRBC
3. Spleen cell response to Con A

D. Humoral Immunity

1. Spleen AFC response to sRBC
2. Serum antibody response to sRBC
3. Spleen cell response to LPS

E. Functional Activity of the Reticuloendothelial System

1. Vascular clearance rate of sRBC
2. Organ uptake of sRBC
3. Chemotaxis, adherence, and phagocytic activity of PEC

F. Bone Marrow

1. DNA synthesis

the reticuloendothelial system, while the sixth set of mice was used to study the number and functional status of PEC. Tables 8–18 show the control values for CD-1 mice in the 90-day studies.

METHODS

Experimental Animals

Although the mouse is currently being used for some toxicological studies, the random bred rat has been the experimental animal most widely used and considered to be the most appropriate for toxicological evaluation of chemicals and drugs. Unfortunately, the physiology of the rat's immune system has not been studied or defined to the same degree as that of the mouse and man. The mouse has been the immunologist's animal of choice because of the availability of inbred strains, which has allowed for the development of specific immune sera and for decreased animal-to-animal variation. In addition, due to the homogeneity of the lymphoid cells derived from genetically similar inbred mice, functional tests can be performed on cell samples pooled from several animals.

As a compromise, the program we have developed employs the random bred CD-1 mouse. Of the three commercial sources of mice we evaluated, only one (Charles River Breeding Laboratories, Wilmington, MA) supplied mice of consistent quality and temperament. This mouse has been used to assess the effects of chemicals on the immune system, and with the increased availability of microassays, has become attractive for the more routine toxicological tests.

Upon arrival, all animals were housed four per cage in plastic shoe box cages containing sawdust bedding (PWI Hardwood Sawdust, Lowville, NY). After the quarantine period (7 days), animals were randomized and were individually earpunched. Acute toxicity studies were performed on animals eight weeks of age.

TABLE 8
Control Values for Body Weight (Grams) of Male and Female CD-1 Mice Used in 90-Day Studies[a]

	Final Weight	Initial Weight	Change in Weight
Males	39.3 ± 4.6	22.2 ± 3.6	17.3 ± 5.7
Females	30.6 ± 3.8	19.5 ± 2.8	11.1 ± 4.5

[a]Values represent the mean ± SD of 288 male and 288 female control mice derived from six 90-day studies. All weights were significantly different ($p < 0.05$) among the six experiments.

TABLE 9
Control Hematology Values for Male and Female CD-1 Mice in 90-Day Studies[a]

Parameter	Male	Female
Hemoglobin (g%)	12.5 ± 2.0	12.3 ± 2.0
Hematocrit (%)	41.3 ± 3.7	41.4 ± 2.0*
Erythrocytes ($10^6/mm^3$)	8.19 ± 1.59	8.51 ± 2.05
Leukocytes ($10^3/mm^3$)	6.42 ± 2.79	6.37 ± 2.94
Platelets ($10^5/mm^3$)	3.78 ± 0.90	3.60 ± 0.81
Prothrombin time (sec)	9.6 ± 0.7	9.7 ± 0.7
APTT	31.1 ± 5.5*	33.1 ± 5.8*
Fibrinogen (mg%)	282 ± 50	211 ± 29
Differential:		
Lymphocytes (%)	67.2 ± 15.8	78.7 ± 9.0
Polymorphonuclears (%)	30.0 ± 15.4	16.1 ± 7.9
Monocytes (%)	3.8 ± 3.3	3.6 ± 2.8
Eosinophils (%)	1.0 ± 1.3*	1.6 ± 1.2*

[a]Hematology values represent the mean ± SD of 131 male and 137 female control CD-1 mice derived from six 90-day studies. Differential values represent the mean ± SD of 88–92 male and female CD-1 mice derived from four subchronic 90-day studies. All values were significantly different ($p < 0.05$) among the six experiments except where indicated by an

The 14-day studies were begun on animals six weeks of age, while four week old animals were used in 90-day studies. Mice exposed by gavage were maintained on Purina Lab Chow and water ad libitum. Those animals exposed to the chemicals in drinking water were maintained on Purina Lab Chow and chemical solutions ad libitum. Control values for body weights of the mice used in the 90-day studies are presented in Table 8. Animal room temperature was maintained between 21 and 24°C and relative humidity between 40-60%. The light-dark cycle was maintained on 12-hr intervals.

If the chemical was water soluble it was placed directly in deionized water. For the chemicals which were water insoluble, a polyethoxylated vegatable oil, emulphor (GAF 620, GAF Corp., New York, NY), was used as the vehicle. Ten percent emulphor in deionized water was used to dissolve chemicals used in gavage studies, while a 1% emulphor solution was used for drinking water studies, if necessary. Solutions for gavaging were prepared fresh daily, while solutions for the drinking water were prepared twice weekly. All solutions were maintained in dark bottles to alleviate possible photodecomposition. Gavaging was done with an 18 gauge stainless steel feeding tube between the hours of 10:00 and 11:00 a.m. Solutions for drinking water studies were placed in 250-500 ml amber colored bottles capped with cork stoppers containing stainless steel sipper tube inserts. All drinking water solutions were analyzed by GLC with head space analysis to determine the stability of the compound over a three- to four-day period at room temperature. Fluid consumption was calculated by weighing the water bottles at the time solutions were initially placed in them, then weighing the bottles again before solutions were changed to determine the amount consumed over the three- or four-day period.

Blood and Tissue Collection, Hematology, and Coagulation Studies

To assure that the maximum amount of blood was obtained and that its quality was applicable for special coagulation tests,

the mice were anesthetized with chloroform. Several anesthetic agents have been tried, and the two that did not perturb any of the parameters examined, including the hepatic microsomal mixed functional oxidase system, were chloroform and sodium brevital. Sodium pentobarbital and hexobarbital perturbed liver microsomal enzymes and ether affected the quality and amount of blood drawn from the animals (data not shown). The blood was collected by cardiac puncture into a 3 ml plastic syringe fitted with a 23 gauge needle. For the hematology and blood coagulation studies, the blood was collected into 3.2% sodium citrate (1:10 citrate to blood). The samples were collected without air bubbles within 30 sec of the puncture. One to two ml of blood were routinely collected by this method. The blood was maintained at 4°C and leukocytes, erythrocytes, and platelets were enumerated on a Coulter counter, Model ZBI. Platelet counts were determined on platelet-rich plasma by a modification of the methods of Bull et al.[5] Leukocyte differentials were evaluated using the standard Wright's-Giemsa staining procedure. Hematocrits were performed on a micro-hematocrit centrifuge and hemoglobins determined by the cyanomethemoglobin method. The plasma was separated from the cellular elements by 800 xg centrifugation at 4°C. The plasma was used to evaluate the status of the coagulation system. Prothrombin times were performed to assess extrinsic activity and activated partial thromboplastin times (APTT) were performed to assess intrinsic activity. These assays were done on a BBL Fibrometer using General Diagnostics reagents (Baltimore, MD). Fibrinogen levels were determined by the kinetic method using Dade Diagnostics reagents (Miami, FL). Control historical hematologic and coagulation data from six 90-day studies are shown in Table 9. The only parameter showing a sex difference was the fibrinogen level, where the females had a 25% lower level than the males.

Immediately after the blood sample was drawn, the liver was examined, removed, trimmed, weighed, and if needed, a section was removed for histopathological examination. The remaining liver tissue was homogenized and a microsomal fraction was prepared for analysis. One femur was removed and the bone marrow collected for analysis. A complete necropsy was then performed and the following organs were removed, trimmed, and weighed: brain, liver, spleen, lungs, thymus, kidneys, and testes. Samples of these tissues were fixed in 10% buffered formalin along with the heart, mesenteric lymph nodes, striated muscle, stomach, ileum, jejunum, colon, bladder, ovaries, and adrenals. The most notable sex differences in organs weights were in the brain and kidney (Table 10). The brain was 28% larger in the female when calculated on a percent body weight basis. The kidney was 17% and 35% larger in the male when calculated on a percent of body weight and organ to brain ratio, respectively.

Preparation of Microsomes

Immediately following sacrifice, livers were removed, weighed, thoroughly rinsed, and homogenized in 9 volumes of ice cold 0.15M KCl - 0.01M potassium phosphate buffer (pH 7.4) using a teflon glass homogenizer, and centrifuged at 9000 xg for 20 min. An aliquot of the supernatant fluid was removed, carefully avoiding the top lipid layer, and recentrifuged at 105,000 xg for 60 min. The resulting pellet was rinsed and resuspended in 0.05M potassium phosphate buffer to obtain a concentration of approximately 2 mg protein per mg of buffer. The protein concentration of the microsomal suspension was determined by the method of Lowry et al.[6], using human serum albumin as the standard.

Drug Metabolism Assays

Substrates were selected as representative of Type I (aminopyrine) and Type II (aniline) cytochrome P450-dependent mixed function oxidase. Historical control data for drug metabolism assays are shown in Table 11.

TABLE 10
Control Organ Weight Values for CD-1 Mice Used in 90-Day Studies[a]

Organ	Weight (mg)	% Body Weight	Organ/Brain
		MALES	
Brain	499 ± 39	1.27 ± 0.14	
Liver	2057 ± 349	5.17 ± 0.53	4.13 ± 0.70
Spleen	167 ± 69	0.42 ± 0.16	0.34 ± 0.14
Lungs	234 ± 36	0.59 ± 0.08*	0.47 ± 0.07
Thymus	45 ± 14	0.11 ± 0.03	0.09 ± 0.03
Kidney	650 ± 102	1.64 ± 0.18*	1.30 ± 0.20
Testes	260 ± 41	0.66 ± 0.10*	0.52 ± 0.08*
		FEMALES	
Brain	497 ± 41	1.63 ± 0.22	-
Liver	1526 ± 242	4.97 ± 0.68	3.10 ± 0.59
Spleen	155 ± 45	0.50 ± 0.13	0.32 ± 0.10
Lungs	216 ± 40	0.71 ± 0.13	0.44 ± 0.09
Thymus	54 ± 16	0.17 ± 0.05	0.11 ± 0.03
Kidney	418 ± 54	1.36 ± 0.16	0.85 ± 0.13

[a]Values represent the mean ± SD of 132 male and 138 female control CD-1 mice derived from six 90-day studies. All values were significantly different ($p < 0.05$) among the six experiments, except where indicated by an asterisk.

Aminopyrine N-demethylase: The methods of Cochin and Axelrod[7] were used to measure the in vitro metabolism of aminopyrine. A 25 ml Ehrlenmeyer flask containing 0.5 ml cofactors and buffer (7.5 µmol $MgCl_2 \cdot 6H_2O$, 17 µmol glucose-6-phos-

TABLE 11
Control Values for Hepatic Microsomal Mixed Functional Oxidase Parameters of Male and Female CD-1 Mice Used in 90-Day Studies

Parameter	Unit	Male	Female
Microsomal Protein	mg/g liver	23.4 ± 3.2	20.4 ± 2.3
Cytochrome P450	nmol/mg protein	1.16 ± 0.259	0.98 ± 0.184
Cytochrome b5	nmol/mg protein	0.41 ± 0.050	0.52 ± 0.079
Aminopyrine N-Demethylase	nmol/mg protein/min	10.6 ± 1.7	13.1 ± 2.1
Aniline Hydroxylase	nmol/mg prot/min	1.60 ± 0.48	1.72 ± 0.37

[a]Values represent the mean ± SD of 44 male and 48 female mice from six 90-day studies. The means of the parameters were significantly different ($p < 0.05$) among the experiments.

phate, 2 µmol NADP, and 1 unit glucose-6-phosphate dehydrogenase in 0.5M potassium phosphate buffer, pH 7.4), 0.5 ml aminopyrine (10 µmol), and 1.0 ml of microsomal suspension (2 mg protein/ml) was prepared. Blanks were prepared similarly, except that aminopyrine was omitted from the flasks. The flasks were shaken for 10 min at 37°C at 120 oscillations per min in a gyratory shaker bath. At the end of the incubation period, the reaction was terminated by the addition of 0.6N perchloric acid. The contents were transferred to centrifuge tubes and centrifuged at 2000 xg for 15 min. One ml of the supernatant fluid was added to 1 ml of double strength Nash reagent (300 g ammonium acetate and 2 ml acetyl acetone per liter H_2O), and the color allowed to develop for 30 min in a 60°C water bath. Samples were cooled immediately and the absorbance at 415 nm was recorded.

Aniline Hydroxylation: The 4-hydroxylation of aniline was determined using a comparable procedure[8], except that 16 µmol of aniline HCl was incubated in the presence of cofactor and

microsomes. The reaction was terminated by addition of 1.0 ml of 20% trichloroacetic acid. One ml of the protein-free supernatant fluid was added to 0.5 ml 10% sodium carbonate, mixed, and added to 1.0 ml of phenol reagent (2% phenol in 0.2N NaOH). The color was allowed to develop at 37°C for 30 min and the absorbance at 630 nm recorded.

Spectral Studies

The methods of Omura and Sato[9] were used to measure the content of microsomal cytochromes P450 and b_5. Historical control data are shown in Table 11.

Cytochrome P450: Microsomes were diluted to 1 mg protein per ml in 0.1M potassium phosphate buffer. Carbon monoxide was gently bubbled through the sample cuvette for 30 sec and a few crystals of sodium dithionite were added to each cuvette. The CO-difference spectrum of the reduced microsomes was recorded from 500-400 nm and cytochrome P450 content was calculated from the absorbance difference (OD 450 nm minus OD 490 nm) using 91 mM^{-1} cm^{-1} as the mM extinction coefficient.

Cytochrome b_5: Microsomes were diluted to 1 mg protein per ml buffer and divided equally into two cuvettes. The sample cuvette was reduced by adding 4.4 µmol NADH in 0.01 ml H_2O. The reduced vs oxidized spectrum was recorded and cytochrome b_5 content calculated from the absorbance difference (OD 423 nm minus OD 409 nm), using 185 mM^{-1} cm^{-1} as the mM extinction coefficient.

Bone Marrow

The status of the bone marrow was evaluated by determining bone marrow cell DNA synthesis. In addition, bone marrow smears were prepared for evaluation if marked changes in the parameter occurred. Bone marrow cells were added to each well of a 96-well microtiter dish at 6×10^5 cells/200 µl. Twenty µl of a solu-

tion containing 0.1 μCi of ^{125}I-Iododeoxyuridine (^{125}I-IUdR; New England Nuclear) in 2 x 10^{-5}M Fluorodeoxyuridine (FUdR; Sigma Chemical Co.) was added to each well. At 60 and 120 min, triplicate wells were harvested and the cells were collected onto filter disks with the aid of a Titertek cell harvester. Filter disks containing the ^{125}I-IUdR incorporated into DNA were radioassayed in a Beckman 300 gamma counter. Figure 2 shows a typical incorporation curve for ^{125}I-IUdR into bone marrow cells. As can be seen, there is a linear incorporation of ^{125}I-IUdR over a 3 hr incubation period. Appropriate studies were performed to assure that ^{125}I-IUdR was incorporated into DNA. Table 12 shows control historical data on bone marrow DNA synthesis.

Serum and Liver Chemistries

The serum enzymes, glutamic-pyruvic transaminase, glutamic-oxaloacetic transaminase, lactate dehydrogenase, and alkaline phosphatase were measured kinetically on the ABA 100 Bichromatic analyzer (Abbott Laboratories, Dallas, TX). These enzymes had been optimized for the mouse as to pH and substrate concentration. A-GENT reagent kits purchased from Abbott Laboratory Diagnostic Division (South Pasadena, CA) were used to determine blood urea nitrogen, total protein, glucose, cholesterol, and calcium, while Pierce reagent kits (Pierce Chemical Co., Rochford, IL) were used to measure phosphorus and creatinine. These chemistries were measured by endpoint analysis on the above-named instrument. Sodium and potassium ions were measured on a flame photometer (Instrumentation Laboratory 443), and chloride ions were measured on a chloridometer (Buchler Instruments). The methods of Jollow et al.[11] were used to measure liver glutathione content. A mixture of 0.5 ml of liver homogenate (described previously) and 0.5 ml of 4% sulfosalicylic acid was centrifuged at 2000 xg for 15 min. Two hundred μl of protein-

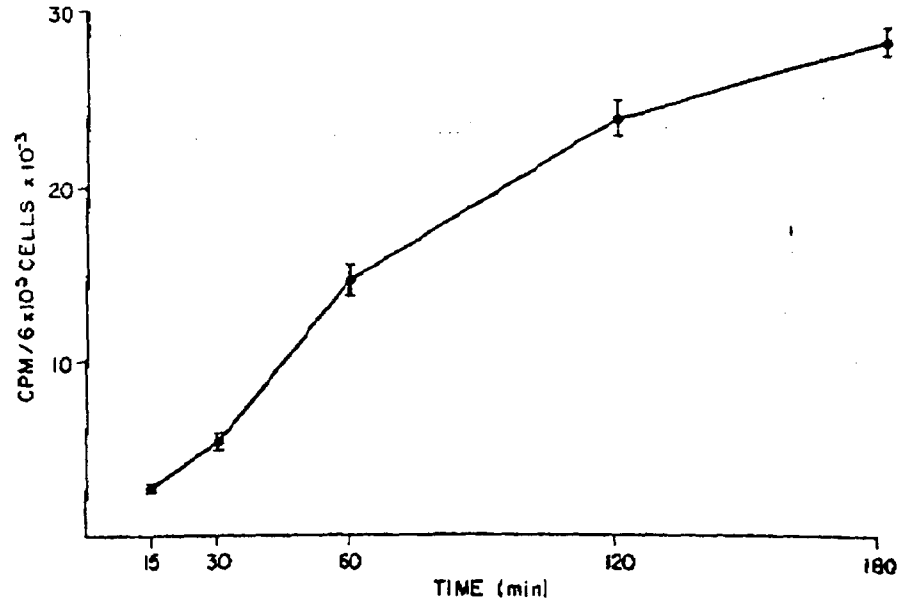

FIG. 2. The time course of incorporation of ^{125}I-IUdR into bone marrow cells over a 180 min period. See Methods for procedure.

TABLE 12
Control Values for Bone Marrow DNA Synthesis from Male and Female CD-1 Mice Used in 90-Day Studies[a]

Parameter	Male	Female
DNA Synthesis (60 min)	16082 ± 4411	17246 ± 4319
DNA Synthesis (120 min)	29563 ± 6655	30224 ± 8823

[a]Values represent the mean ± SD of 60 male and female mice from six 90-day studies. There were no significant differences ($p < 0.05$) among the six experiments.

free supernatant fluid were added to 2.0 ml of 0.4 mM Ellman's Reagent (5,5'-dithiobis-2-nitrobenzoic acid) in 0.1M potassium phosphate buffer, pH 8.0. Ten min after mixing, the absorbance at 412 nm was recorded and compared to the absorbance of glutathione standards. Baseline historical control data for the serum and liver chemistries from the 90-day studies are shown in Table 13.

Cell-Mediated Immunity

The functional status of both the afferent and efferent arms of cellular immunity was evaluated by measuring a DTH response to sRBC. Sheep erythrocyte sensitization was done on Day 0 in the left hind footpad (LFP) with 1×10^8 sRBC in a volume of 20 µl. Four days following sensitization, the mice were challenged in the same footpad with 4×10^8 sRBC in a volume of 40 µl. Seventeen hr following the challenge, mice were injected intravenously with 0.3 ml of ^{125}I-human serum albumin (^{125}I-HSA; 80,000 cpm/0.1 ml; Mallinckrodt). Two hr later, the mice were sacrificed by cervical dislocation and both hind feet were removed at the ankle joint and radioassayed in a gamma counter. It has been shown that ^{125}I-HSA will extravasate into the edematous area produced by a DTH response (Paranjpe and Boone[11]). The right hind footpad (RFP) served as a control for background infiltration of ^{125}I-HSA. A group of mice which were unsensitized but challenged as above acted as unsensitized controls to determine non-specific swelling. Results are expressed as a stimulation index (SI), which was calculated are follows:

$$SI = \frac{\text{LFP sensitized}}{\text{RFP sensitized}} - \text{mean } \frac{\text{LFP unsensitized.}}{\text{RFP unsensitized}}$$

Evaluation of cell-mediated immunity using the footpad assay requires both the afferent arm (antigen recognition and processing, blastogenesis) and the efferent arm (lymphokine production,

TABLE 13
Control Serum and Liver Chemistry Values in Male and Female CD-1 Mice Used in 90-Day Studies[a]

Parameter	Unit	Male	Female
LDH	IU/L	664 ± 233	606 ± 193
SGPT	IU/L	44.6 ± 24.4	36.5 ± 18.6
SGOT	IU/L	84.4 ± 38.9	92.0 ± 28.2
SAP	IU/L	44.1 ± 22.6	62.4 ± 29.7
Glutathione	µmol/g liver	8.97 ± 1.79	7.85 ± 1.66
BUN	mg%	27.7 ± 6.9	22.8 ± 5.2
Protein	g	7.13 ± 1.21	7.56 ± 1.08
Glucose	mg%	160 ± 32	137 ± 24
Cholesterol	mg%	167 ± 98	120 ± 77
Creatinine	mg%	0.42 ± 0.19	0.50 ± 0.23
Phosphorus	mEq/L	8.40 ± 2.01	8.05 ± 2.28
Calcium	mEq/L	12.0 ± 2.1	11.7 ± 1.6
Sodium	mEq/L	155 ± 10	156 ± 11
Chloride	mEq/L	108 ± 12	109 ± 14
Potassium	mEq/L	7.30 ± 0.94	6.90 ± 0.95

[a]Values represent the mean ± SD derived from 70-143 control CD-1 mice used in six 90-day studies. LDH=lactate dehydrogenase, SGPT=serum glutamic-pyruvic transaminase, SGOT=serum glutamic-oxaloacetic transaminase, SAP=serum alkaline phosphatase, BUN=blood urea nitrogen. The means of each parameter were significantly different ($p < 0.05$) among the six experiments.

increased vascular permeability) to function adequately. If an experimental chemical decreases the extravasation of ^{125}I-HSA, it may be evaluated as immunosuppressive in the cell-mediated response. However, this may not necessarily be true if the compound has anti-inflammatory properties. To determine if a chemical affects the afferent arm of the cellular response, prolifer-

ation of the popliteal lymph node cells to sRBC was measured. Additional groups of animals were used for this assay. They were subjected to the same procedure as were the DTH animals, except that 1 1/2 hr after challenge they received 20 μg/kg FUdR intraperitoneally, and 2 hr after challenge they received 1 μCi of ^{125}I-IUdR intravenously. ^{125}I-HSA was not administered to these animals. These mice were sacrificed 24 hr after challenge and both popliteal lymph nodes were removed and counted in a gamma counter. Non-specific proliferation was corrected for and an SI was calculated as described for the footpad assay. Historical control data for both footpad swelling and popliteal lymph node proliferation are shown in Table 14.

Lymphocyte Responsiveness

The mitogens employed were Con A, a T cell mitogen in the mouse, and LPS, a B cell mitogen. A 0.05 ml volume of RPMI 1640 medium containing either 1, 5, or 10 μg of Con A or 1, 5, or 20 μg of LPS was placed in flat-bottom microtiter wells and frozen at -70°C. When needed, plates were removed from the freezer and thawed at room temperature. No differences were observed between mitogen plates frozen and those freshly prepared. To detect a maximal mitogen response and to detect any shifts in the dose response curve, three concentrations of mitogen were routinely used.

Spleens were aseptically removed from mice and single cell suspensions prepared by pushing spleens through sterile 60-mesh wire screens into RPMI 1640 media supplemented with 5% heat-inactivated fetal calf serum (HIFCS), 2 mM L-glutamine, 100 μg/ml penicillin and 1 μg/ml streptomycin. The cell concentration was adjusted to 5 x 10^6 cells/ml in RPMI medium supplemented with 10% HIFCS, L-glutamine, and penicillin-streptomycin. One tenth ml of the cell suspension was placed in thawed microtiter wells containing mitogen; additionally, wells were set up without mitogen to act as background controls.

TABLE 14
Control Values for Cell-Mediated Immune Response as Measured by DTH and Popliteal Lymph Node Proliferation to sRBC in Male and Female CD-1 Mice Used in 90-Day Studies[a]

Stimulation Index	Male	Female
Footpad Swelling	4.02 ± 2.05	4.61 ± 2.15
Popliteal Lymph Node Proliferation	18.0 ± 12.1	12.0 ± 6.7*

[a]Footpad swelling values represent the mean ± SD of 103 male and 104 female mice derived from six 90-day studies. A value of 1.95 for males and 2.07 for females has been subtracted from each animal to correct for non-specific swelling as described in Methods. Popliteal lymph node data were obtained from three 90-day studies using 29 males and 32 females. To correct for non-specific lymph node proliferation, a value of 1.1 for males and 1.3 for females was subtracted from each animal. The means of each parameter were significantly different ($p < 0.05$) among the experiments, except where indicated by an asterisk.

The plates were then placed in a 10% CO_2, 37°C humidified incubator. After 48 hr, 0.05 ml of a radiolabeled solution was added to each well. This solution contained ^{125}I-IUdR (4 μCi/ml) and FUdR (4 x 10^{-6}M). The plates were reincubated for 18-20 hr, at which time the cells were collected on filter disks using a Titertek cell harvester. The filter disks were then counted in a gamma counter. There are two ingredients which were essential for this assay to work: heat-inactivated fetal calf serum and FUdR.

Historical control data for lymphocyte responsiveness to Con A and LPS are shown in Table 15.

Humoral Immunity

Hemolytic Plaque Assay: The method used in our laboratory to detect AFC was the Cunningham modification of the Jerne

TABLE 15
Control Values for Spleen Lymphocyte Responsiveness to Con A and LPS in Male and Fenale CD-1 Mice Used in 90-Day Studies[a]

Parameter	Male (cpm/5 x 10^5 cells)	Female (cpm/5 x 10^5 cells)
No mitogen	7778 ± 4959	10179 ± 6601
Con A (1 µg)	97654 ± 58212	118697 ± 53177
Con A (5 µg)	165116 ± 68393	195746 ± 62703
Con A (10 µg)	55920 ± 74316	51333 ± 74816
LPS (1 µg)	51064 ± 33406	83905 ± 42547
LPS (5 µg)	53138 ± 33179	90994 ± 41820
LPS (20 µg)	49571 ± 34632	83618 ± 44002

[a]Values represent the mean cpms ± SD derived from 75 male and female control CD-1 mice used in five 90-day studies. The means for each parameter were significantly different ($p < 0.05$) among the five experiments.

plaque assay[12]. IgM AFC were enumerated 4 and 5 days following intraperitoneal immunization with 4 x 10^8 sRBC. Data are presented as AFC/10^6 spleen cells and also as AFC/spleen. Historical control data for IgM AFC from six 90-day studies are shown in Table 16.

Hemagglutination: Mice were immunized intraperitoneally with 1 x 10^9 sRBC on day 0. On day 7, blood was collected into 3.2% sodium citrate as previously described. A sample of the plasma was removed and heat inactivated at 56°C for 30 min. Serial 1:1 dilutions were made in phosphate buffered saline in round bottom 96-well microtiter plates to a final volume of 0.1 ml. To each well was added 0.1 ml of a 0.5% sRBC solution. The plates were covered and placed in a 37°C humidified incubator.

TABLE 16
Control Values for Humoral Immunity as Measured by Spleen IgM AFC and Hemagglutination in Male and Female CD-1 Mice Used in 90-Day Studies[a]

Parameter	Male Day 4	Male Day 5	Female Day 4	Female Day 5
Spleen Weight	189 ± 61	177 ± 38	196 ± 44	183 ± 55*
Spleen Cell No. (x 10^8)	1.67 ± 0.59	1.54 ± 0.44	1.68 ± 0.52	1.65 ± 0.52
IgM AFC/Spleen (x 10^{-5})	3.15 ± 1.53	1.64 ± 0.94	3.54 ± 1.47	1.92 ± 0.73
IgM AFC/10^6 Spleen Cells	1851 ± 488	1016 ± 401	2114 ± 737	1200 ± 412
Hemagglutination Titer (Log_2)	9.39 ± 0.89		9.63 ± 1.0	

[a]Values represent the mean ± SD derived from 70 fenale and male CD-1 mice used in six subchronic 90-day studies. Hemagglutination titers were derived from 48 mice used in four subchronic 90-day studies. The assays were carried out as described in Methods. The means for each parameter were significantly different ($p < 0.05$) among the experiments, except where indicated by an asterisk.

Two hr later, the plates were removed and placed on a magnifying mirror to determine at which serum dilution no agglutination occurs. The historical control data from four 90-day studies evaluating hemagglutination are shown in Table 16.

Macrophage Function

Functional Ability of the Reticuloendothelial System (RES): Five ml of freshly drawn sRBC (5 x 10^9 cells/ml) were radiolabeled with 1 mCi of sodium chromate-51 (New England Nuclear) in a 37°C shaker bath for 30 min. After chromation, the sRBC

were washed with Alsever's solution until the supernatant was virtually radioactivity free. Unlabeled sRBC (5×10^9/ml) were added to the labeled cells until the hematocrit was approximately 12%. The resulting cpms were 200,000/0.1 ml. The sRBC were refrigerated and used the following day. Before use, the cells were washed to remove any free chromium which was release overnight, and resuspended in phosphate buffered saline (PBS).

Mice used for evaluation of RES activity were weighed and placed in shoebox cages maintained at 39°C. At 0 time, 0.1 ml of labeled particle/10 g body weight was injected intravenously. Ten µl blood samples were taken from the tip of the tail at 2, 4, 6, 8, 10, and 15 min. At 60 min, mice were sacrificed by decapitation and selected organs collected. The blood samples were put into 1 ml of distilled water and radioassayed. Blood clearance was expressed as the phagocytic index, which was determined by the slope of the clearance curve.

Figure 3 shows the time course of particle uptake from the selected organs. For a routine study, mice were sacrificed at the plateau period (60 min after injection). Liver, spleen, lungs, thymus, and kidneys were removed, weighed, and counted in a gamma counter. Organ distribution was expressed as percent organ uptake and cpms/mg tissue (specific activity). Control values from six 90-day studies are given in Table 17.

Recruitability of PEC: Mice were injected intraperitoneally with 1 ml of 10% Brewer's thioglycolate on day 0. On day 5, the mice were sacrificed by cervical dislocation, and the peritoneal cavity was flushed with 10 ml of minimum essential medium (MEM). The cells were centrifuged at 300 xg for 10 min and then suspended in fresh MEM. A sample was removed and enumerated on a Coulter counter in the presence of a lysing agent to determine the number of PEC recruited.

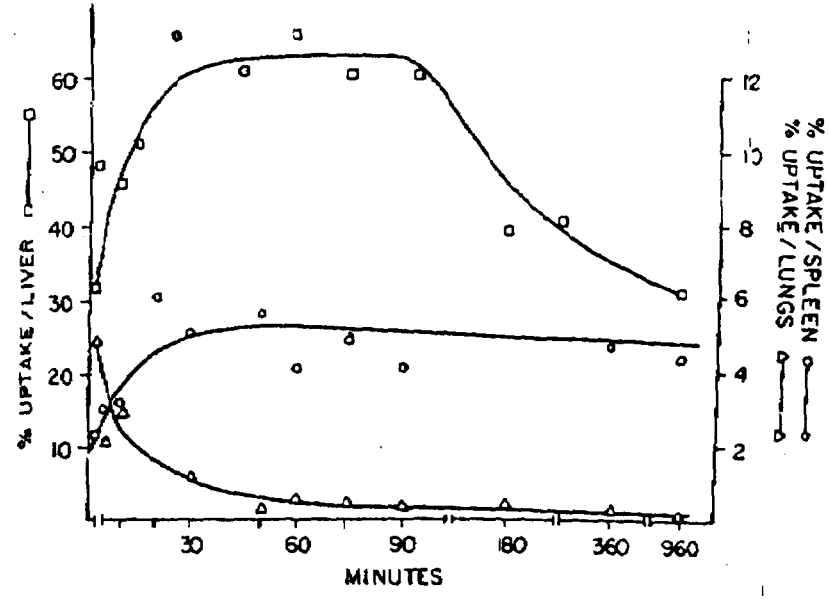

FIG. 3. The time course of particle uptake in liver, spleen, and lung. See Methods for procedure.

TABLE 17
Control Values for the Functional Activity of the RES as Measured by Vascular and Organ Clearance of Cr-51 Labeled sRBC in Male and Female CD-1 Mice Used in 90-Day Studies[a]

Parameter	Male		Female	
	% Uptake	cpm/mg	% Uptake	cpm/mg
Phagocytic Index	0.10 ± 0.05		0.11 ± 0.07	
Liver	49.6 ± 11.1	217 ± 66	57.6 ± 10.6	247 ± 69
Spleen	8.81 ± 4.08	552 ± 299	10.6 ± 6.2	493 ± 332
Lung	1.31 ± 1.11	43 ± 46	1.27 ± 0.93*	36 ± 28
Thymus	.006 ± .002	4 ± 3*	.010 ± .007	6 ± 6
Kidney	2.29 ± 1.10*	30 ± 17	2.27 ± 0.79	36 ± 13

[a]Values represent the mean ± SD derived from 95 control CD-1 mice used in six 90-day studies. The means for each parameter were significantly different ($p < 0.05$) among the six experiments, except where indicated by an asterisk.

Phagocytic Activity of PEC: PEC were collected, resuspended, and counted as described above. Cell concentrations were adjusted to 2 x 10^5/ml and 1 ml samples added to each well of a 24-well Costar dish. Plates were incubated for one hr in a humidified CO_2 incubator (37°C). The medium was decanted and the adherent cells were washed with MEM. To each well was added 5 µl of chromated sRBC (~100,000 cpm) prepared as described above, which had been opsonized with mouse IgG. The adhered PEC and ^{51}Cr sRBC were incubated for 10, 20, 30, and 45 min. The plates were then washed once with distilled water and then twice with medium to remove any adhered sRBC. One M NaOH (1 ml) was then added to each well to dissolve the PEC and then the contents of the wells were counted in a gamma counter.

Adherence of PEC: PEC were collected, resuspended, and counted as described previously. Cell concentrations were adjusted to 2 x 10^5/ml and 1 ml samples were placed in each well of a 24-well Costar plate. The plates were incubated for 18-24 hr in a 37°C humidified CO_2 incubator. The cells were then washed extensively, scraped from the plates, and counted on a Coulter counter.

Chemotaxis of PEC: Chemotaxis of PEC was measured in modified blind well Boyden chambers using 13 mm Sartorius membrane filters as described by Laskin et al.[13] Varying numbers of cells (1 to 2 x 10^6 cells/ml) were placed in the upper portion of the chamber, with vehicle or chemoattractant (endotoxin activated mouse serum) in the lower portion. After incubation at 37°C for 4 hr, the filters were removed, rinsed in methanol, and fixed in formalin until stained. They were then placed on glass slides, stained with hematoxylin, rinsed, dipped in 0.1% NH_4OH for 1 min, and rinsed in distilled water. After processing through 95% ethanol, absolute ethanol, and isopropanol, the filters were cleared in xylene, mounted on glass slides, and dried. Using a Nikon microscope interfaced with an Artec Model 980 Image Analyzer, 20 microgrid fields were counted.

TABLE 18
Control Values for PEC Parameters in Male and Female CD-1 Mice Used in 90-Day Studies[a]

Parameter	Male	Female
Recruitable PEC (x 10^{-7})	1.0 ± .11	2.0 ± .17
Adherent PEC (x 10^{-5})	2.6 ± .08	2.6 ± .06
Chemotaxis (cells/field)		
No Chemoattractant	22.6 ± 6	26.0 ± 6
EAMS 1:10	167 ± 27	68 ± 10
Phagocytosis of Ab-^{51}Cr sRBC (cpm)		
10 minutes	596 ± 99	354 ± 58
20 minutes	1086 ± 182	449 ± 66
30 minutes	1420 ± 216	604 ± 83
45 minutes	1693 ± 233	705 ± 112

[a]Values represent the mean ± SD of 32 male and female CD-1 mice, derived from three 90-day studies. EAMS = endotoxin activated mouse serum, Ab-^{51}Cr sRBC = ^{51}Cr labeled sRBC opsonized with mouse IgG.

Historical control values for PEC parameters are given in Table 18.

Statistical Evaluation

If a one way analysis of variance of the means showed study to study differences, a Duncan's Multiple Range Test was performed[14]. Values which differ from study to study at $p < 0.05$ were considered statistically significant. Each of the values represents the mean ± SE.

DISCUSSION

We have chosen to approach the problem of evaluating the effects of chemicals on the lymphoreticular system from a toxi-

cological viewpoint. Acute, 14- and 90-day studies were utilized for each chemical. The acute study was used to aid in selecting doses for subsequent studies and suggesting possible toxicological target sites. In the 90-day studies, both male and female mice were used. The outbred CD-1 mouse was our animal of choice and, as an outbred strain, the increased animal-to-animal variation was acceptable. Due to the random genetic background of the mice, many of the parameters monitored did not fall into a normally distributed response. Most notable is the variation in the number of AFC responding to sRBC in control animals. There appear to be at least three distinct populations of animals: low responders, medium responders, and high responders. In an attempt to circumvent this problem, a large number of mice was used for each treatment dose and for each parameter evaluated.

The effects of a chemical on the immune response were not studied independently, but along with a multitude of standard toxicological parameters. This results in a more complete overview of the chemical's effect. In evaluating the effects of a compound, specifically on the immune system, not just one, but several assays were used in assessing humoral immunity, cell-mediated immunity, and macrophage function.

It has been our experience that historical controls can only be used as a guide. As repeatedly pointed out in this paper, experiments using the same strain of mice, obtained from the same supplier, and handled in an identical manner by the same people results in control values which will differ significantly from experiment to experiment at the $p < 0.05$ level as determined by a one way analysis of variance. Thus, historical controls are only beneficial in establishing a data base. When comparing data for safety assessment or evaluation of drug effects, one must compare the treatment groups to the appropriate concurrent controls used in the actual study.

Since the assays outlined here can be used to evaluate the status of the immune system in a manner which is quantifiable and reproducible, the assessment of the immunotoxicity of chemicals can be easily incorporated into routine toxicity testing. These methods have been used in this laboratory to evaluate the toxicity and immunotoxicity of some halogenated hydrocarbons found to be drinking water contaminants[15,16,17]. The following four reports use these same approaches to assess the general toxicity and immunotoxicity of two other drinking water contaminants, trans-1,2-dichloroethylene (DCE) and 1,1,2-trichloroethane (TCE).

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REFERENCES

1. L.D. Koller, Some immunological effects of lead, cadmium, and methylmercury, Drug Chem. Toxicol., 2, 99 (1979).

2. P.T. Thomas and R.D. Hinsdall, The effect of perinatal exposure to tetrachlorodibenzo-p-dioxin on the immune response of young mice, Drug Chem. Toxicol., 2, 77 (1979).

3. J.G. Vos, M.J. VanLogten, J.G. Kreeftenberg, P.A. Steerenberg, and W. Kruizinga, Effect of hexachlorobenzene on the immune system of rats following combined pre- and postnatal exposure, Drug Chem. Toxicol., 2, 61 (1979).

4. J.H. Goodnight, Probit Procedure, in SAS User's Guide, 1979 Edition, J.T. Helwig and K.A. Council, eds., SAS Institute Inc., Raleigh, NC, 1978, p. 357.

5. B.S. Bull, M.A. Schneiderman, and G. Brecher, Platelet counts with the Coulter Counter, Am. J. Clin. Path., 44, 678 (1965).

6. O.H. Lowry, N.J. Rosebrough, A.L. Farr, and R.J. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem., 193, 265 (1951).

7. J. Cochin and J. Axelrod, Biochemical and pharmacological changes in the rat following chronic administration of morphine, nalorphine, and normorphine, J. Pharmacol. Exp. Ther., 125, 105 (1959).

8. Y. Imai, A. Ito, and R. Sato, Evidence for biochemically different types of vesicles in the hepatic microsomal fraction, J. Biochem., 60, 417 (1966).

9. T. Omura and R. Sato, The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature, J. Biol. Chem., 239, 2370 (1964).

10. D.J. Jollow, J.R. Mitchell, N. Zampiglione, and J.R. Gillette, Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite, Pharmacology (Basel), 11, 151 (1974).

11. M.S. Paranjpe and C.W. Boone, Delayed hypersensitivity to simian virus 40 tumor cells in BALB/c mice demonstrated by a radioisotopic footpad assay, J. Natl. Cancer Inst., 48, 563 (1972).

12. A.J. Cunningham and A. Szenberg, Further improvements in the plaque technique for detecting single antibody-forming cells, Immunology, 14, 599 (1968).

13. D.L. Laskin, J.D. Laskin, I.B. Weinstein, and R.A. Carchman, Induction of chemotaxis in mouse peritoneal macrophages by phorbol ester tumor promoters, Cancer Res., 41, 1923 (1981).

14. J.P. Sall, Duncan Procedure, in SAS User's Guide, 1979 Edition, J.T. Helwig and K.A. Council, eds., SAS Institute Inc., Raleigh, NC, 1979, p. 191.

15. A.N. Tucker, V.M. Sanderrs, D.W. Barnes, T.J. Bradshaw, K.L. White, Jr., L.E. Sain, J.F. Borzelleca, and A.E. Munson, Toxicology of trichloroethylene in the mouse, Toxicol. Appl. Pharmacol., 62, 351 (1982).

16. V.M. Sanders, A.N. Tucker, K.L. White, Jr., B.M. Kauffmann, P. Hallett, R.A. Carchman, J.F. Borzelleca, and A.E. Munson, Humoral and cell-mediated immune status in mice exposed to trichloroethylene in the drinking water, Toxicol. Appl. Pharmacol., 62, 358 (1982).

17. A.E. Munson, L.E. Sain, V.M. Sanders, B.M. Kauffmann, K.L. White, Jr., D.G. Page, D.W. Barnes, and J.F. Borzelleca, Toxicology of organic drinking water contaminants: trichloromethane, bromodichloromethane, dibromochloromethane and tribromomethane, Environ. Health Persp., 46, 117 (1982).

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TOXICOLOGY OF 1,1,2-TRICHLOROETHANE IN THE MOUSE

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ABSTRACT

1,1,2-Trichloroethane (TCE) was administered to male and female CD-1 mice to evaluate its effect on standard toxicological parameters. Following determination of the acute LD50 (378 mg/kg in males and 491 mg/kg in females), and a 14-day range-finding study, a 90-day drinking water study was performed in which the doses consumed were 4.4, 46, and 305 mg/kg for males and 3.9, 44, and 384 mg/kg for females. The liver was a target of TCE toxicity in both sexes as demonstrated by dose-dependent alterations in hepatic microsomal enzyme activities and serum enzyme levels. The erythroid element of the female mice was also affected, as indicated by significantly decreased hematocrit and hemoglobin levels.

INTRODUCTION

1,1,2-Trichloroethane (TCE) is one of the organic chemicals detected in drinking water supplies at levels from 0.1 to 8.5 μg/l[1]. It also has been detected in industrial effluents at levels of 5.4 mg/l[2]. TCE is used as a solvent for fats, oils, waxes, and resins and in the synthesis of organic chemicals[3].

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Also, in water treatment, small amounts of TCE can be formed during chlorination[3].

All of the toxicological data available on TCE are derived from short-term exposure (e.g., a single i.p. injection), except for the gavage studies performed by the NCI for carcinogenicity bioassays[4]. Acute oral toxicity studies on TCE using both male and female mice have not been reported. Klassen and Plaa[5] reported that the i.p. LD50 of TCE in male mice is 242 mg/kg. Smyth et al.[6] reported the oral LD50 in rats to be 580 mg/kg and the dermal LD50 in rabbits to be 3730 mg/kg.

Other toxicological studies on TCE evaluated the hepatic and renal effects following a single i.p. injection. Hepatotoxicity and nephrotoxicity have been investigated primarily in mice and dogs. Cellular infiltration, hepatocyte vacuolation, elevation of SGPT, and prolonged bromosulfophthalein retention have been observed in mice[5]. Increases in SGPT levels in mice treated i.p. with TCE were even greater when the mice receive isopropyl alcohol or acetone[7]. In dogs, mild centrilobular necrosis, slight subcapsular necrosis, and vacuolization of centrilobular hepatocytes have been observed in association with increases in SGPT levels[8]. In experiments designed to examine nephrotoxicity, the presence of hyaline droplets, nuclear pyknosis, hydropic degeneration, increased eosinophilia, tubular necrosis with karyolysis, and loss of the epithelium of convoluted tubules have been reported in mice in association with altered phenolsulfonphthalein (PSP) excretion[5]. In dogs, tubular necrosis and decreased PSP excretion have been observed after i.p. injection of TCE[8].

The toxicological investigations presented in this manuscript were undertaken to provide data that would assist in the hazard assessment of this chemical in the drinking water. After the LD50 was determined in mice by the oral route, a 14-day range-finding study was performed to provide information necessary for designing a 90-day study in which the compound was administered in the drinking water. This manuscript reports the general toxicological status of mice exposed orally to TCE, while the following manuscript will discuss the immune status of these mice in relation to the toxicological findings[9].

MATERIALS AND METHODS

Details of the methods for the general toxicological assays and historical controls have been fully described by White et al. in the first article of this volume[10].

Chemical

1,1,2-Trichloroethane (TCE), lots 070177 and 061197, was purchased from Aldrich Chemical Co. (Milwaukee, WI). The purity was given as 95%. The structure was verified by infrared spectrometry and the purity verified by gas chromatography. No stabilizers or preservatives were present with this chemical.

Chemical Administration

Solutions of TCE were prepared fresh daily for the acute and 14-day studies and the appropriate concentrations were administered by gavage in a volume of 0.01 ml/g of body weight to achieve the desired dose. Solutions were prepared on the day of administration in a 1:9 (v/v) solution of emulphor (GAF Corp., New York, NY) and deionized water, and maintained in dark glassware at 4°C until used. In the acute study, TCE was administered as a single dose to male and female mice after an 18 hr fast, at seven doses from 200 to 600 mg/kg. In the 14-day study, concentrations of TCE were prepared so that each mouse received 1/100 and 1/10 the LD50 daily for 14 days (3.8 and 38 mg/kg). For the 90-day study, TCE was diluted in deionized water to achieve con-

centrations of 0.02, 0.2, and 2.0 mg/ml and administered in the drinking water. These concentrations resulted in a delivery of approximately 1/100, 1/10 and 1/1 the LD50. The solutions were maintained at room temperature in amber-colored bottles with stainless steel spouts fitted through cork stoppers. Water bottles were changed twice weekly. Less than 10% of the TCE was lost during the three or four days between bottle changes, as measured by GLC with head space analysis.

Fluid consumption during the 90-day study was estimated by the change in fluid weight over the three or four day period when solutions were changed. Twelve cages of control mice and eight cages of each treatment group for each sex were used to estimate fluid consumption. Chemical exposure was calculated from fluid consumption and is reported as mg TCE/kg/day. Fluid consumption is reported as ml/kg/day.

Statistical Evaluation

If a one-way analysis of variance of the means showed treatment effects, a Duncan's Multiple Range Test was performed[11]. Values which differ from vehicle control at $p < 0.05$ were considered statistically significant. Each of the values represents the mean ± SE.

RESULTS

Acute Toxicity

The acute toxicity of TCE was performed to determine doses for use in the 14-day range-finding studies and to define a toxicological profile following exposure by the gastrointestinal route. The LD50's for TCE administered as a single gavage to male and female mice were 378 and 491 mg/kg, respectively (Table 1). All mice receiving doses greater than or equal to 450 mg/kg

TABLE 1
Acute Toxicity (mg/kg) of TCE in CD-1 Mice[a]

Sex	Number of Mice	LD50	Log Probit Slope
Male	56	378 (344 - 408)	17.6
Female	56	491 (452 - 550)	13.7

[a]CD-1 male and female mice were fasted for 18 hr prior to a single gavage of TCE. The LD50 with corresponding 95% confidence limits (numbers in parentheses) and the slope were calculated according to the Log Probit Procedure[12].

became sedated within an hour, while 10% of these mice lost their righting reflex. At the end of the fourth hour, most ani mals had recovered from the anesthetic effect of TCE and did no appear to be in acute distress. Most deaths occurred within 24 hr, and no deaths occurred after 48 hr. Observations following chemical administration were performed over a 14-day period. A the time of death, necropsies revealed a dose-dependent irrita tion of the upper gastrointestinal tract, with 100% of the mic at the 500 and 600 mg/kg dose levels showing gastric irritatio Fifty to 75% of the animals in each group had pale livers, and up to 25% demonstrated lung damage as manifested be reddened o hemorrhagic areas.

Fourteen Day Study

No significant differences were observed between the body weights of TCE-exposed mice and control mice over the 14-day gavage period, and no exposure-related mortality occurred (data not shown). Brain, thymic, and testicular weights increased significantly in male mice dosed at 38 mg/kg when expressed on

TABLE 2
Body and Organ Weights of Male CD-1 Mice Exposed by Gavage for Fourteen Days to TCE[a]

Exposure Group	Body Wt. (g)	Organ	Wt. (mg)	Percent Body Wt.	Organ/ Brain
Vehicle[b]	27.9 ± 1.1	Brain	408 ± 15	1.48 ± 0.06	-
		Liver	1699 ± 92	6.09 ± 0.23	4.21 ± 0.24
		Spleen	158 ± 14	0.56 ± 0.04	0.39 ± 0.03
		Lungs	202 ± 14	0.71 ± 0.03	0.48 ± 0.03
		Thymus	67 ± 4	0.24 ± 0.02	0.16 ± 0.01
		Kidneys	458 ± 33	1.62 ± 0.07	1.11 ± 0.05
		Testes	194 ± 9	0.70 ± 0.03	0.48 ± 0.02
3.8 mg/kg	28.1 ± 0.6	Brain	414 ± 10	1.48 ± 0.04	-
		Liver	1870 ± 68	6.67 ± 0.22*	4.52 ± 0.12
		Spleen	145 ± 7	0.52 ± 0.03	0.35 ± 0.02
		Lungs	199 ± 10	0.71 ± 0.03	0.48 ± 0.02
		Thymus	70 ± 6	0.25 ± 0.02	0.17 ± 0.01
		Kidneys	460 ± 21	1.63 ± 0.06	1.11 ± 0.04
		Testes	190 ± 10	0.68 ± 0.04	0.46 ± 0.02
38 mg/kg	29.8 ± 0.6	Brain	453 ± 9*	1.52 ± 0.03	-
		Liver	1917 ± 53	6.43 ± 0.09	4.52 ± 0.12
		Spleen	154 ± 8	0.52 ± 0.02	0.34 ± 0.02
		Lungs	225 ± 8	0.75 ± 0.02	0.50 ± 0.02
		Thymus	84 ± 4*	0.28 ± 0.01	0.19 ± 0.01
		Kidneys	489 ± 16	1.64 ± 0.04	1.08 ± 0.03
		Testes	228 ± 8*	0.76 ± 0.02	0.50 ± 0.02

[a]Values represent mean ± SE derived from 12 mice per group.
[b]Vehicle = 10% emulphor
*Significantly different from vehicle at $p < 0.05$.

a total organ weight basis (Table 2). When expressed as a percent of body weight, or as an organ to brain ratio, no significant changes occurred. Hematological, coagulation, SGPT, and BUN values were all within the range of vehicle control values (Table 3). Lactic dehydrogenase activity was depressed 21% below control in the 38 mg/kg group.

TABLE 3
Selected Hematological, Coagulation, and Serum Chemistry Value for Male CD-1 Mice Exposed by Gavage for 14 Days to TCE[a]

Parameter	Vehicle[b]	3.8 mg/kg TCE	38 mg/kg T
Hematocrit (%)	41.4 ± 0.7	41.9 ± 0.6	41.4 ± 0.7
Hemoglobin (g%)	11.8 ± 0.4	11.6 ± 0.4	13.1 ± 0.5
Leukocytes ($10^3/mm^3$)	8.25 ± 0.68	7.31 ± 0.61	7.47 ± 0.6
Fibrinogen (mg%)	283 ± 7	280 ± 7	275 ± 9
Prothrombin Time (sec)	8.3 ± 0.1	8.3 ± 0.1	8.3 ± 0.1
LDH[c] (IU/L)	1059 ± 47	954 ± 55	835 ± 62*
SGPT[c] (IU/L)	88.3 ± 9.5	82.8 ± 12.9	59.2 ± 5.0
BUN[c] (mg%)	27.3 ± 1.0	26.6 ± 1.6	25.2 ± 0.9

[a]Values represent mean ± SE derived from 11-12 mice per group.
[b]Vehicle = 10% emulphor
[c]LDH = lactate dehydrogenase; SGPT = serum glutamic-pyruvic transaminase; BUN = blood urea nitrogen.
*Significantly different from vehicle at $p < 0.05$.

Ninety Day Study

On the foundation of the data from the acute toxicity and 14-day range-finding studies, concentrations of TCE in the drinking water solutions were designed to deliver daily doses equivalent to, and higher than, those administered by gavage. The desired doses were 3.8, 38, and 380 mg/kg (1/100, 1/10 and 1/1 the LD50) and the concentrations of chemical calculated to deliver these doses in the drinking water were 0.02, 0.2, and 2.0 mg/ml. The 380 mg/kg dose was included as the data from th 14-day exposure indicated that higher doses may be tolerated. The calculated time-weighted average doses delivered based on fluid consumption and body weights were 4.4, 46, and 305 mg/kg for the male mice and 3.9, 44, and 384 mg/kg for the female mic (Table 4). Monthly time-weighted averages for fluid consumptio are also shown in Table 4. On a ml/kg basis, there was a 30% decrease in fluid consumption in male mice drinking the highest

TABLE 4
Time-Weighted Averages of Consumption of Fluid by CD-1 Mice Receiving TCE in the Drinking Water for 90 Days[a]

Concentration (mg/ml)	Days of Study[b] 0–30	31–60	61–90	Cumulative (Days 0–90) ml/kg/day	mg/kg/day[c]	ml/mouse day[d]
			MALES			
0	252 ± 5	242 ± 4	225 ± 4	240 ± 5	0	8.2 ± 0.1
0.02	203 ± 7	241 ± 5	209 ± 5	219 ± 4	4.4 ± 0.1	7.8 ± 0.1
0.20	226 ± 5	254 ± 7	207 ± 5	228 ± 6	46 ± 1.2	7.6 ± 0.2
2.00	162 ± 4*	152 ± 5*	139 ± 6*	153 ± 9*	305 ± 18.2	4.5 ± 0.2*
			FEMALES			
0	230 ± 5	171 ± 4	172 ± 4	193 ± 3	0	5.2 ± 0.1
0.02	192 ± 6	198 ± 5	197 ± 4	195 ± 5	3.9 ± 0.1	5.3 ± 0.1
0.20	206 ± 6	265 ± 6	197 ± 4	222 ± 7	44 ± 1.3	6.0 ± 0.1
2.00	184 ± 4	212 ± 5	186 ± 5	192 ± 5	384 ± 9.5	5.3 ± 0.1

[a]Values represent mean ± SE derived from 48 mice in the control (deionized water) group and 32 mice in the other groups. Means were calculated for each month of the study, as well as for the entire 90 days.
[b]Average ml fluid/kg body weight/day for the period indicated.
[c]Average mg of TCE/kg body weight/day.
[d]Average ml fluid/mouse/day.
*Significantly different from control at $p < 0.05$.

concentration of TCE over the 90-day period. However, there was no TCE-related change in fluid consumption for the female mice.

The growth curves for mice exposed to TCE reflect their fluid consumption pattern. Male mice demonstrated a concentration-dependent reduction in weight gain (Figure 1). Although the animals were randomized at the beginning of exposure and an analysis of variance among the cages showed no difference in body weights, there was an early (day 3) reduction in weight for the male mice receiving the highest concentration of TCE. After this weight loss, these mice resumed a normal growth pattern,

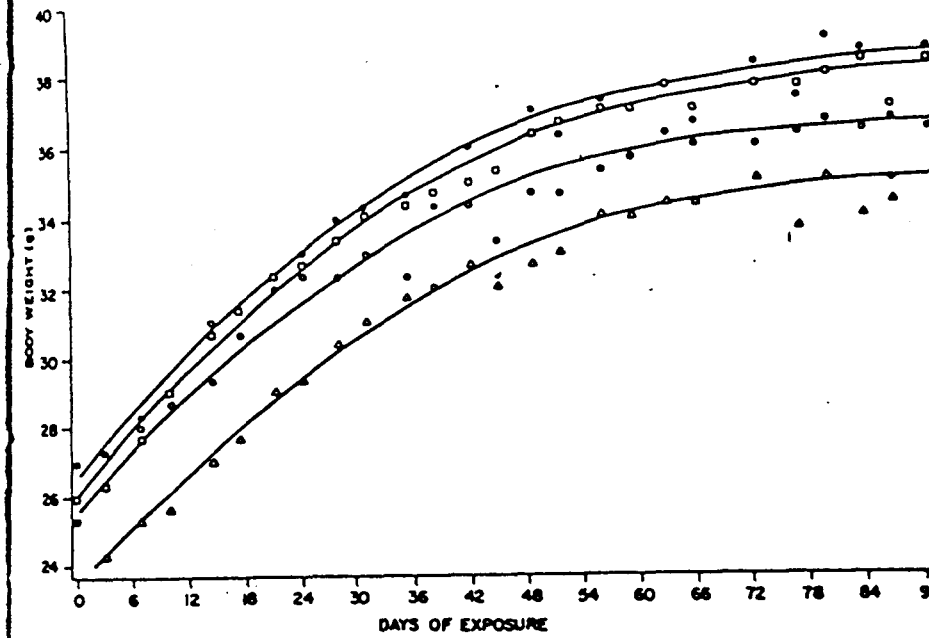

FIG. 1. Growth chart of male CD-1 mice exposed to TCE in the drinking water: • deionized water; □ 4.4 mg/kg delivered, 0.02 mg/ml in water; o 46 mg/kg delivered, 0.2 mg/ml in water; △ 305 mg/kg delivered, 2.0 mg/ml in water. There were 48 mice in the deionized water group, and 32 mice in each of the TCE-exposed groups.

although they always lagged behind the other mice due to the initial reduction. Over the 90-day exposure period, male mice exposed to deionized water gained 12.2 ± 0.5 g (mean ± SE), while the male mice exposed to 0.02 and 0.2 mg/ml TCE gained 11.8 ± 0.5 and 11.2 ± 0.6 g, respectively. Male mice exposed to 2.0 mg/ml gained 7.9 ± 1.1 g, which was significantly less than the control group. In contrast to the males, the weight gain of female mice exposed to TCE was not altered from control (Figure 2).

Organ weights for male and female mice are shown in Tables 5 and 6. In males, there were no significant changes in weights for the brain, spleen, lungs, and thymus. On a mg basis, liver

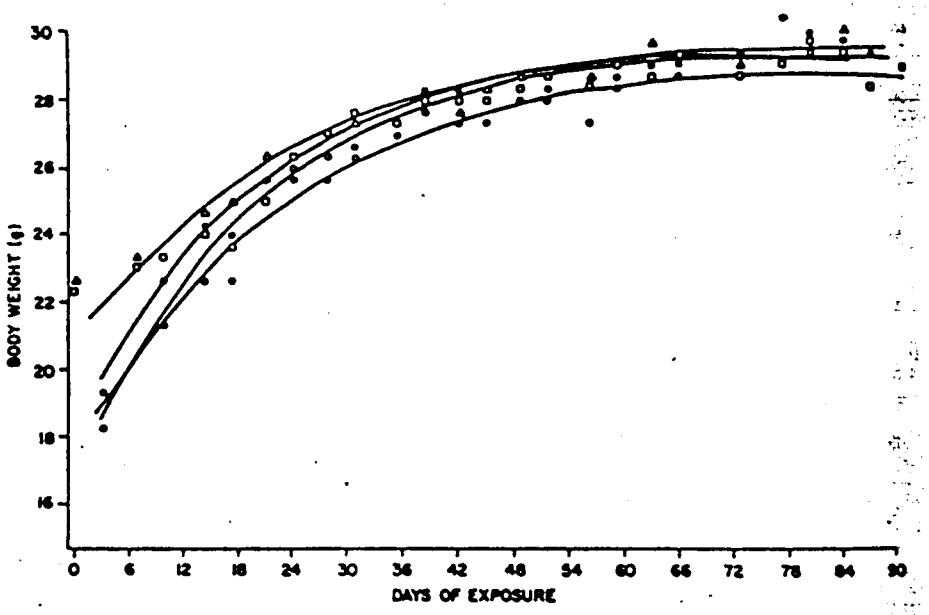

FIG. 2. Growth chart of female CD-1 mice exposed to TCE in the drinking water: • deionized water; □ 3.9 mg/kg delivered, 0.02 mg/ml in water; o 44 mg/kg delivered, 0.2 mg/ml in water; △ 384 mg/kg delivered, 2.0 mg/ml in water. There were 48 mice in the deionized water group, and 32 mice in each of the TCE-exposed groups.

weights in male mice exposed to 0.2 and 2.0 mg/ml TCE were 11 and 12% smaller than the control group. This decreased liver weight was proportional to the decreased body weight; therefore, no significant changes could be measured on a percent of body weight basis. Similarly, kidneys weights were decreased 12 and 16% on a mg basis for the groups receiving 0.2 and 2.0 mg/ml. Again, no change from control was evident on a percent of body weight basis. Testicular weights of males exposed to the two highest concentrations increased 11 and 12% above control, but only on a percent of body weight basis. Females responded differently in that the group receiving 2.0 mg/ml TCE had a 32% increase in absolute liver weight and a 26% increase when expressed as per-

TABLE 5
Body and Organ Weights of Male CD-1 Mice Exposed to TCE in the Drinking Water for 90 Days[a]

Parameter	Deionized Water	TCE 0.02 mg/ml	TCE 0.2 mg/ml	TCE 2.0 mg/ml
Body Wt. (g)	39.7 ± 0.7	41.0 ± 0.5	37.6 ± 1.1	35.8 ± 1.0*
Brain (mg)	490 ± 7	498 ± 8	480 ± 11	468 ± 14
(% body wt.)	(1.24)	(1.22)	(1.29)	(1.31)
Liver (mg)	2125 ± 57	2152 ± 58	1889 ± 90*	1875 ± 45*
(% body wt.)	(5.33)	(5.24)	(5.01)	(5.25)
Spleen (mg)	190 ± 25	175 ± 13	152 ± 13	137 ± 8
(% body wt.)	(0.48)	(0.42)	(0.41)	(0.38)
Lungs (mg)	239 ± 6	237 ± 7	241 ± 12	228 ± 5
(% body wt.)	(0.60)	(0.58)	(0.64)	(0.64)
Thymus (mg)	39 ± 2	39 ± 3	45 ± 4	42 ± 4
(% body wt.)	(0.10)	(0.09)	(0.12)	(0.12)
Kidneys (mg)	660 ± 17	648 ± 24	580 ± 13*	555 ± 22*
(% body wt.)	(1.66)	(1.58)	(1.55)	(1.55)
Testes (mg)	255 ± 6	259 ± 6	263 ± 8	258 ± 7
(% body wt.)	(0.64)	(0.63)	(0.71)*	(0.72)*

[a]Values represent mean ± SE derived from 23 mice in the deionized water group and 14–16 mice in the other groups.
*Significantly different from control at $p < 0.05$.

cent of body weight. Spleen and kidney weights of females exposed to the highest concentration were significantly increased on a mg basis only.

There were few TCE exposure-related alterations in the hematological or blood coagulation parameters in the male mice (Tables 7 and 8). Male mice exposed to the highest concentration of TCE had decreased percent of polymorphonuclear leukocytes and

TABLE 6
Body and Organ Weights of Female CD-1 Mice Exposed to TCE in the Drinking Water for 90 Days[a]

Parameter	Deionized Water	TCE 0.02 mg/ml	TCE 0.2 mg/ml	TCE 2.0 mg/ml
Body Wt. (g)	29.9 ± 0.4	29.8 ± 0.6	30.0 ± 0.8	31.2 ± 0.6
Brain (mg) (% body wt.)	528 ± 4 (1.77)	521 ± 6 (1.76)	522 ± 9 (1.75)	516 ± 6 (1.66)*
Liver (mg) (% body wt.)	1368 ± 41 (4.57)	1410 ± 32 (4.74)	1318 ± 54 (4.39)	1806 ± 82* (5.76)*
Spleen (mg) (% body wt.)	132 ± 6 (0.44)	134 ± 4 (0.45)	117 ± 8 (0.38)	156 ± 12* (0.49)
Lungs (mg) (% body wt.)	213 ± 5 (0.71)	220 ± 8 (0.74)	215 ± 8 (0.72)	225 ± 6 (0.72)
Thymus (mg) (% body wt.)	49 ± 2 (0.16)	42 ± 3 (0.14)	41 ± 3 (0.14)*	50 ± 3 (0.16)
Kidneys (mg) (% body wt.)	392 ± 9 (1.31)	386 ± 15 (1.30)	380 ± 13 (1.27)	430 ± 10* (1.38)

[a]Values represent mean ± SE derived from 23 mice in the deionized water group and 16 mice in the other groups.
*Significantly different from control at $p < 0.05$.

increased percent of lymphocytes. This shift was probably due to a minor biting problem among the male mice in the control and low dose groups, which resulted in a lower than normal percent of lymphocytes and a higher than normal percent of polymorphonuclear cells in these groups.

Hemoglobin and hematocrit values of female mice exposed to 2.0 mg/ml TCE decreased 6 and 5% ($p < 0.05$), as compared to the mice receiving deionized water. Erythrocytes also decreased, but not at the $p < 0.05$ level (Table 9). Platelet counts increased in

TABLE 7
Hematology of Male CD-1 Mice Exposed to TCE in the Drinking Water for 90 Days[a]

Parameter	Deionized Water	TCE 0.02 mg/ml	TCE 0.2 mg/ml	TCE 2.0 mg/ml
Hematocrit (%)	39.8 ± 0.9	40.1 ± 1.3	40.3 ± 0.6	38.6 ± 0.9
Hemoglobin (g%)	13.8 ± 0.4	13.5 ± 0.4	13.4 ± 0.3	13.2 ± 0.3
Erythrocytes ($10^6/mm^3$)	7.70 ± 0.24	7.71 ± 0.35	7.96 ± 0.21	7.01 ± 0.19
Leukocytes ($10^3/mm^3$)	6.79 ± 0.55	5.63 ± 0.48	5.42 ± 0.44	6.57 ± 0.66
Platelets ($10^5/mm^3$)	4.08 ± 0.17	3.85 ± 0.29	3.80 ± 0.22	4.05 ± 0.28
Fibrinogen (mg%)	292 ± 16	265 ± 20	273 ± 17	262 ± 14
Prothrombin Time (seconds)	9.2 ± 0.1	9.3 ± 0.2	9.4 ± 0.1	9.3 ± 0.1
APTT[b] (seconds)	31.8 ± 1.5	30.8 ± 0.9	29.0 ± 1.1	30.8 ± 0.9

[a]Values represent mean ± SE derived from 23 mice in the deionized water group and 14-16 mice in the other groups.
[b]APTT = activated partial thromboplastin time.

all groups, but not in a dose-dependent fashion. A 48% increase in leukocyte number was also observed in the females. However, the biological significance of this is questionable, primarily because the leukocyte count of the deionized water control group is at the low end of the historical control data accumulated in our laboratory (6.37 ± 2.94)[10]. Fibrinogen levels increased in females exposed to all three exposure levels, while prothrombin times decreased in the two highest exposure groups.

TABLE 8
Differential Cell Counts of CD-1 Mice Exposed to TCE in the Drinking Water for 90 Days[a]

Exposure Group	Percent of Total White Blood Cells			
	Lymphocytes	Polymorphonuclears	Monocytes	Eosinophils
		MALES		
Deionized Water	53.3 ± 2.9	40.4 ± 2.9	5.1 ± 0.6	1.2 ± 0.3
0.02 mg/ml	58.2 ± 4.2	37.2 ± 4.2	3.6 ± 0.7	0.9 ± 0.4
0.2 mg/ml	61.8 ± 3.8	34.3 ± 3.3	2.9 ± 0.8	1.1 ± 0.3
2.0 mg/ml	72.2 ± 1.8*	22.4 ± 1.6*	4.2 ± 0.7	1.2 ± 0.4
		FEMALES		
Deionized Water	81.0 ± 1.6	13.1 ± 1.2	4.0 ± 0.5	1.9 ± 0.5
0.02 mg/ml	74.4 ± 2.2*	20.1 ± 2.2*	3.8 ± 0.8	1.7 ± 0.4
0.2 mg/ml	78.7 ± 2.1	17.0 ± 2.4	3.3 ± 0.7	0.9 ± 0.3
2.0 mg/ml	81.1 ± 2.3	10.8 ± 2.0	5.7 ± 0.7	2.3 ± 0.7

[a]Values represent mean ± SE derived from 22-24 mice in the deionized water group and 14-16 mice in the other groups.
*Significantly different from control at $p < 0.05$.

Sera were analyzed for selected enzymes, ions, and other marker molecules, including glucose, blood urea nitrogen, and creatinine. In all, 14 serum chemistry parameters were evaluated (Tables 10 and 11). In the control mice, glucose, cholesterol, SGOT, SGPT, and SAP were all slightly elevated compared to our historical controls[10]. The serum chemistry values altered in males which could be considered exposure-related were the 28%

TABLE 9
Hematology of Female CD-1 Mice Exposed to TCE in the Drinking Water for 90 Days[a]

Parameter	Deionized Water	TCE 0.02 mg/ml	TCE 0.2 mg/ml	TCE 2.0 mg/ml
Hematocrit (%)	41.0 ± 0.3	40.8 ± 0.5	40.8 ± 0.6	39.1 ± 0.5*
Hemoglobin (g%)	14.0 ± 0.1	13.9 ± 0.3	14.2 ± 0.2	13.1 ± 0.3*
Erythrocytes ($10^6/mm^3$)	8.36 ± 0.17	8.39 ± 0.31	8.34 ± 0.20	7.75 ± 0.19
Leukocytes ($10^3/mm^3$)	4.34 ± 0.24	5.06 ± 0.37	4.88 ± 0.23	6.42 ± 0.44*
Platelets ($10^5/mm^3$)	3.76 ± 0.13	4.21 ± 0.11*	4.12 ± 0.16	4.19 ± 0.16*
Fibrinogen (mg%)	188 ± 3	225 ± 6*	211 ± 7*	209 ± 7*
Prothrombin Time (seconds)	9.8 ± 0.1	9.5 ± 0.2	9.1 ± 0.2*	8.0 ± 0.1*
APTT[b] (seconds)	33.1 ± 1.2	33.1 ± 1.4	31.8 ± 0.8	36.5 ± 1.5

[a]Values represent mean ± SE derived from 23 mice in the deionized water group and 16 mice in the other groups.
[b]APTT = activated partial thromboplastin time.
*Significantly different from control at $p < 0.05$.

increase in cholesterol levels and the 61% increase in SAP activity in mice exposed to the highest concentration of TCE. The exposure-related alterations in the females included a 36% increase in cholesterol levels and a 63% increase in SGPT activity at the highest dose level, and SGOT and SAP increases at all dose levels (not dose dependent).

Liver glutathione levels decreased dose dependently in males exposed to the two highest concentrations of TCE by 16 and 28%

TABLE 10
Serum Chemistry and Liver Glutathione Levels of Male CD-1 Mice Exposed to TCE in the Drinking Water for 90 Days[a]

Parameter	Deionized Water	TCE 0.02 mg/ml	TCE 0.2 mg/ml	TCE 2.0 mg/ml
Calcium (mg%)	11.4 ± 0.2	12.0 ± 0.2	12.4 ± 0.2*	12.1 ± 0.2*
Phosphorus (mg%)	6.90 ± 0.20	6.91 ± 0.26	6.63 ± 0.28	6.57 ± 0.26
Sodium (mEq/L)	146 ± 1	154 ± 3*	149 ± 1	152 ± 2*
Chloride (mEq/L)	112 ± 2	111 ± 2	115 ± 5	121 ± 4*
Potassium (mEq/L)	7.49 ± .21	8.32 ± .21*	6.84 ± .21*	6.74 ± .18*
Protein (g%)	7.85 ± .23	7.77 ± .24	8.03 ± .23	8.25 ± .27
Glucose (mg%)	183 ± 10	171 ± 11	185 ± 11	207 ± 15
Cholesterol (mg%)	305 ± 15	304 ± 21	342 ± 16	392 ± 22*
BUN (mg%)	34.1 ± 1.5	29.0 ± 1.0*	31.8 ± 1.3	31.7 ± 1.0*
LDH (IU/L)	638 ± 55	589 ± 91	736 ± 45	676 ± 64
SGPT (IU/L)	62.3 ± 8.4	40.6 ± 3.4	39.9 ± 4.3*	53.1 ± 8.3
SGOT (IU/L)	116.5 ± 14.0	91.4 ± 8.6	79.0 ± 4.2*	104.2 ± 10.5
SAP (IU/L)	53.0 ± 4.2	52.2 ± 7.1	57.6 ± 8.6	85.1 ± 9.5*
Creatinine (mg%)	0.34 ± .02	0.25 ± .03*	0.31 ± .02	0.38 ± .01
Glutathione (μmol/g liver)	10.83 ± .44	9.96 ± .52	9.08 ± .25*	7.77 ± .51*

[a]Values represent mean ± SE derived from 24 mice in the deionized water group and 14–16 mice in the other groups. Glutathione levels were derived from 8 mice per group.
*Significantly different from control at $p < 0.05$.
BUN = blood urea nitrogen; LDH = lactate dehydrogenase; SGPT = serum glutamic-pyruvic transaminase; SGOT = serum glutamic-oxaloacetic transaminase; SAP = serum alkaline phosphatase

TABLE 11
Serum Chemistry and Liver Glutathione Levels of Female CD-1 Exposed to TCE in the Drinking Water for 90 Days[a]

Parameter	Deionized Water	TCE 0.02 mg/ml	TCE 0.2 mg/ml	TCE 2.0 m
Calcium (mg%)	11.4 ± 0.2	12.1 ± 0.3*	11.9 ± 0.2	11.7 ±
Phosphorus (mg%)	7.17 ± 0.26	7.02 ± 0.34	7.22 ± 0.30	7.04 ±
Sodium (mEq/L)	166 ± 1	163 ± 3	162 ± 1	166 ±
Chloride (mEq/L)	110 ± 3	115 ± 4	116 ± 3	114 ±
Potassium (mEq/L)	7.66 ± .15	6.96 ± .26*	6.51 ± .26*	7.22 ±
Protein (g%)	7.58 ± .13	8.26 ± .25*	7.95 ± .21	8.21 ±
Glucose (mg%)	159 ± 7	175 ± 7	167 ± 9	168 ±
Cholesterol (mg%)	219 ± 8	223 ± 12	247 ± 17	298 ±
BUN (mg%)	25.0 ± 0.9	32.4 ± 1.6*	25.8 ± 1.4	29.2 ±
LDH (IU/L)	662 ± 42	708 ± 66	768 ± 59	806 ±
SGPT (IU/L)	36.9 ± 2.2	49.8 ± 5.6	46.8 ± 7.0	60.2 ±
SGOT (IU/L)	83 ± 5	117 ± 12*	107 ± 7*	107 ±
SAP (IU/L)	56.8 ± 3.4	78.2 ± 4.0*	73.2 ± 5.9*	68.8 ±
Creatinine (mg%)	0.38 ± .02	0.40 ± .02	0.40 ± .03	0.40 ±
Glutathione (μmol/g liver)	8.00 ± .17	8.33 ± .18	7.59 ± .22	9.07 ±

[a]Values represent mean ± SE derived from 24 mice in the deionized water group and 16 mice in the other groups. Gluta levels were derived from 8 mice per group.
*Significantly different from control at $p < 0.05$.
BUN = blood urea nitrogen; LDH = lactate dehydrogenase; SGPT serum glutamic-pyruvic transaminase; SGOT = serum glutamic-oxaloacetic transaminase; SAP = serum alkaline phosphatase

TABLE 12
Hepatic Microsomal Activities in Male CD-1 Mice Exposed to TCE in the Drinking Water for 90 Days[a]

Parameter	Deionized Water	TCE 0.02 mg/ml	TCE 0.2 mg/ml	TCE 2.0 mg/ml
Microsomal Protein (mg/g liver)	23.8 ± 1.0	23.9 ± 1.5	25.2 ± 0.9	22.8 ± 1.1
Cytochrome P-450 (nmol/mg protein)	1.129 ± .069	1.048 ± .046	1.110 ± .072	1.030 ± .054
Cytochrome b5 (nmol/mg protein)	.392 ± .018	.389 ± .019	.424 ± .021	.387 ± .034
Aminopyrine N-demethylase (nmol/mg/min)	11.8 ± 0.7	11.5 ± 0.7	10.9 ± 0.6	10.8 ± 0.5
Aniline Hydroxylase (nmol/mg/min)	2.03 ± .12	1.72 ± .06	2.23 ± .09	2.45 ± .25

[a]Values represent mean ± SE derived from 8 mice per group.

(Table 10). Female mice exposed to the highest concentration of TCE showed a 13% elevation in glutathione levels (Table 11).

The effects of 90 day exposure to TCE upon hepatic microsomal activities are shown in Tables 12 and 13. In males, there were no perturbations of microsomal protein, cytochrome P-450 and b5 content. Microsomal enzymes that demethylate aminopyrine or hydroxylate aniline were not affected (Table 12). Changes did occur with some of these parameters in the female mice (Table 13). Cytochrome P-450 content and aniline hydroxylase activity were reduced dose dependently, while microsomal protein, cytochrome b5, and aminopyrine demethylase activity were unaffected.

TABLE 13
Hepatic Microsomal Activities in Female CD-1 Mice Exposed to TCE in the Drinking Water for 90 Days[a]

Parameter	Deionized Water	TCE 0.02 mg/ml	TCE 0.2 mg/ml	TCE 2.0 mg/ml
Microsomal Protein (mg/g liver)	22.4 ± 0.9	22.1 ± 0.6	23.8 ± 0.6	22.6 ± 0.
Cytochrome P-450 (nmol/mg protein)	1.080 ± .029	1.014 ± .031	.978 ± .029*	.798 ± .0
Cytochrome b5 (nmol/mg protein)	.491 ± .010	.454 ± .014	.464 ± .015	.494 ± .0
Aminopyrine N-demethylase (nmol/mg/min)	13.6 ± 0.9	12.7 ± 0.5	12.4 ± 0.5	13.0 ± 0.
Aniline Hydroxylase (nmol/mg/min)	1.74 ± .04	1.57 ± .07	1.50 ± .08*	0.92 ± .0

[a]Values represent mean ± SE derived from 8 mice per group.
*Significantly different from control at $p < 0.05$.

DISCUSSION

There is increased interest in the chlorinated hydrocarbons, particularly the haloalkanes and haloalkenes, since they have been identified in finished drinking water supplies[3]. With the discovery of previously unknown chemical dumps and potential contamination of drinking water supplies, there is a need for more definitive toxicological data assessing the potential hazard of these contaminants.

The LD50's for male and female mice for TCE administered by the oral route (378 mg/kg for males and 491 mg/kg for females) are slightly higher than those reported by Klassen and Plaa[5] for the i.p. route in male mice (242 mg/kg). These values are

similar to the LD50 for 1,2-dichloroethane (489 and 413 mg/kg for male and female mice, respectively)[13]. Deaths from acute administration of TCE and 1,2-dichloroethane appear to result from depression of the central nervous system. In contrast, trichloroethylene and trans-1,2-dichloroethylene are much less toxic than TCE with the LD50's for males and females for trichloroethylene being 2402 and 2443 mg/kg[14], and being 2122 and 2391 mg/kg, respectively, for trans-1,2-dichloroethylene[15].

In the 14-day range-finding study conducted on male mice, no definitive toxicity could be detected at doses of 3.8 and 38 mg/kg. Thus, in the 90-day study, concentrations in the drinking water were established to deliver 3.8, 38, and 380 mg/kg. The highest dose would represent an LD50 dose for males, if given as a bolus. In preliminary preference taste tests, mice did not avoid the chemical (data not shown); however, in the 90-day study, males, but not females, consumed less fluid when TCE was present (Table 4). This decreased fluid consumption in males may have caused a decrease in their growth rate. Based on fluid consumption, the males consumed a time-weighted average dose of 308 mg/kg/day at the highest dose level of TCE. Female mice showed no preference for deionized water over TCE-containing water, as seen in fluid consumption data and in their growth pattern.

The only significant dose-related effects due to 90 days of exposure to TCE in males was a decreased liver glutathione level and an elevation in SAP. There was also an elevation in serum cholesterol; however, since the control group showed levels higher than those usually found in our historical controls[10], the interpretation of these data is difficult. The reduction in liver glutathione and the elevation of SAP indicate an effect on the liver of males. However, there were no elevations of SGOT or SGPT in males, which would be expected if an exposure of this duration resulted in liver damage. It may be that the consumption of liver glutathione protected the liver and possibly other target tissues of male mice from the effects of this chemical. This was not the case for females. Not only were glutathione levels not decreased, but they were elevated along with SGOT, SGPT, SAP, fibrinogen levels, and liver weight. There was also a decrease in cytochrome P-450 content and aniline hydroxylase activity, which provides the basis for potential synergistic effects with other drugs and chemicals.

In these studies, the lowest effect level seen in females was 0.2 mg/ml (44 mg/kg), which resulted in a reduction of cytochrome P-450 levels and aniline hydroxylase activity. The no adverse effect level was 0.02 mg/ml (3.9 mg/kg). In males, the lowest effect level was 0.2 mg/ml (46 mg/kg), which resulted in reduction of liver glutathione, and the no adverse effect level was 0.02 mg/ml (4.4 mg/kg).

TCE appears to have a short half life and little bioaccumulation considering the fact that mice given the LD50 in the drinking water daily for 90 days did not result in any deaths. Furthermore, few other toxic signs were noted, among them being loss of body weight and changes in liver parameters. This indicates that TCE may not be a serious hazard in the chronic situation and that it may be more important to monitor acute exposure to this compound.

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REFERENCES

1. W.E. Coleman, R.D. Lingg, R.G. Melton, and F.C. Kopfler, The occurrence of volatile organics in five drinking water sup-

plies using gas chromatography/mass spectrometry, in Identification and Analysis of Organic Pollutants in Water, L.H. Keith ed., Ann Arbor Sci., Ann Arbor, MI, 1976, pp. 305-327.

2. Eurocorp-Cast, A comprehensive list of polluting substances which have been identified in various fresh waters, effluent discharges, aquatic animals and plants and bottom sediments, 2nd ed., Comm. of Eur. Communities, Luxembourg, 1976, p. 56.

3. U.S. Envir. Prot. Agency, Preliminary assessment of suspected carcinogens in drinking water, Report to Congress, Office of Toxic Substances, USEPA, Washington, DC, 1975.

4. National Cancer Institute, Bioassay of 1,1,2-trichloroethane for possible carcinogenicity, Technical Report Series No. 74, DHEW Publishing, Washington, DC, 1978.

5. C.D. Klassen and G.L. Plaa, Relative effects of various chlorinated hydrocarbons on liver and kidney function in mice, Toxicol. Appl. Pharmacol., 9, 139 (1966).

6. H.F. Smyth, Jr., C.P. Carpenter, C.S. Weil, V.C. Pozzani, J.A. Stregel, and V.S. Nycum, Range-finding toxicity data: List VII, Amer. Industr. Hyg. Assoc. J., 30, 470 (1969).

7. G.J. Traiger and G.L. Plaa, Chlorinated hydrocarbon toxicity. Potentiation by isopropyl alcohol and acetone, Arch. Environ. Health, 28, 276 (1974).

8. C.D. Klassen and G.L. Plaa, Relative effects of various chlorinated hydrocarbons on liver and kidney functions in dogs, Toxicol. Appl. Pharmacol, 10, 119 (1967).

9. V.M. Sanders, K.L. White, Jr., L.E. Sain, G.M. Shopp, Jr., J.F. Borzelleca, R.A. Carchman, and A.E. Munson, Humoral and cell-mediated immune status of mice exposed to 1,1,2-trichloroethane, Drug Chem. Toxicol., _____ (1985).

10. K.L. White, Jr., V.M. Sanders, D.W. Barnes, A.N. Tucker, L.E. Sain, G.M. Shopp, Jr., R.A. Carchman, and A.E. Munson, Immunotoxicological investigations in the mouse. General approach and methods, Drug Chem. Toxicol., _____ (1985).

11. J.P. Sall, Duncan Procedure, in SAS User's Guide, 1979 Edition, J.T. Helwig and K.A. Council, eds., SAS Institute, Inc., Raleigh, NC, 1979, pp. 191-194.

12. J.H. Goodnight, Probit Procedure, in SAS User's Guide, 1979 Edition, J.T. Helwig and K.A. Council, eds., SAS Institute, Inc., Raleigh, NC, 1979, pp. 357-360.

13. A.E. Munson, V.M. Sanders, K.A. Douglas, L.E. Sain, B.M. Kauffmann and K.L. White, Jr., In vivo assessment of immunotoxicology, Environ. Health Persp., 43, 41 (1982).

14. A.N. Tucker, V.M. Sanders, D.W. Barnes, T.J. Bradshaw, K.L. White, Jr., L.E. Sain, J.F. Borzelleca, and A.E. Munson, Toxicology of trichloroethylene in the mouse, Toxicol. Appl Pharmacol., 62, 351 (1982).

15. D.W. Barnes, V.M. Sanders, K.L. White, Jr., G.M. Shopp, Jr. L.E. Sain, J.F. Borzelleca, and A.E. Munson, Toxicology of trans-1,2-dichloroethylene in the mouse, Drug Chem. Toxicol _____ (1985).

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BIOASSAY OF 1,1,2-TRICHLOROETHANE FOR POSSIBLE CARCINOGENICITY

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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health

REPORT ON THE BIOASSAY OF 1,1,2-TRICHLOROETHANE
FOR POSSIBLE CARCINOGENICITY

CARCINOGENESIS TESTING PROGRAM
DIVISION OF CANCER CAUSE AND PREVENTION
NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH

FOREWORD: This report presents the results of the bioassay of 1,1,2-trichloroethane conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This is one of a series of experiments designed to determine whether selected environmental chemicals have the capacity to produce cancer animals. Negative results, in which the test animals do not have a in greater incidence of cancer than control animals, do not necessarily mean the test chemical is not a carcinogen because the experiments are conducted under a limited set of circumstances. Positive results demonstrate that the test chemical is carcinogenic for animals under the conditions of the test and indicate a potential risk to man. The actual determination of the risk to man from animal carcinogens requires a wider analysis.

CONTRIBUTORS: This bioassay of 1,1,2-trichloroethane was conducted by Hazleton Laboratories America, Inc., Vienna, Virginia, initially under direct contract to the NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design was determined by the NCI Project Officers, Dr. J. H. Weisburger (1,2) and Dr. E. K. Weisburger (1). The principal investigators for the contract were Dr. M. B. Powers (3), Dr. R. W. Voelker (3), Dr. W. A. Olson (3,4) and Dr. W. M. Weatherholtz (3). Chemical analysis was performed by Dr. C. L. Guyton (3,5) and the analytical results were reviewed by Dr. N. Zimmerman (6); the technical supervisor of animal treatment and observation was Ms. K. J. Petrovics (3).

Histopathologic examinations were performed by Dr. R. H. Habermann (3) and reviewed by Dr. R. W. Voelker (3) at the Hazleton Laboratories America, Inc., and the diagnoses included in this report represent the interpretation of these pathologists. Histopathology findings and reports were reviewed by Dr. R. L. Schueler (7).

Compilation of individual animal survival, pathology, and summary tables was performed by EG&G Mason Research Institute (8); the statistical analysis was performed by Mr. W. W. Belew (6) and Dr. J.